MORPHOLOGY AND PHYSIOLOGY OF AREAS OF LANGERHANS IN SOME VERTEBRATES.

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Plates XII, XIII, XIV, and XV.

Probably no organ or tissue of the body has been the subject of more thought or investigation than have the areas of Langerhans, especially during the last few years, and yet there are many questions that still remain unanswered. Since a complete review of the extensive literature on this subject would extend this paper beyond the limits that seem desirable, and since relatively exhaustive reviews of the literature have recently been given by Schulze, Ssobolew, Sauerbeck, and many others, it seems unnecessary to do more than call attention briefly to articles which deal with those phases of the subject with which my own work has been especially concerned.

As is well known, the islands were first described in 1869 by Langerhans and later by Laguesse, Opie, Diamare, v. Ebner, Gutmann, and many others. As to their development, Hansemann believes that they are mesenchymal in origin, having no connection at any time with the pancreatic tubules, but arising from the interstitial tissue. Küster and Pearce, investigating human embryos, and Laguesse from the study of sheep embryos arrived at the conclusion that the islands develop from the same anlage as the pancreatic glandular structures. These authors differ, however, somewhat in the details of their conclusions, since Küster states that the islands arise from the ducts, the anlagen, when first differentiated, showing central nuclei, homogeneous protoplasm with a tendency to a band-like arrangement of the cells and having capillaries between the bands of cells.

Pearce, on the other hand, believes that they develop from the pancreatic tubules and are at first solid and later vascularized, a reticulum developing still later. Laguesse concludes that they have originally the same entodermal origin as the glandular acini, but later they are transitory structures, developing from the tubules and changing back into the tubules.

On the general structure of these areas, investigators are practically agreed and the description given by v. Ebner expresses briefly the generally accepted ideas concerning their structure. He states that as a rule, but especially in man, the Langerhans islands are surrounded by a connective tissue sheath, which may be interrupted in places so that island cells and acinal cells are in immediate contact. The arrangement of the cells is peculiar because of their close relation to the large blood vessels, which he regards as venous. These are surrounded on all sides by the cells, like the blood capillaries of the liver lobule, the cells resting directly on the walls of the large blood capillaries without any membrana propria or adventitia capillaris between them. In man, occasionally, the area is divided into smaller compartments by strands of connective tissue and within these compartments again the capillary walls rest directly on the epithelial cells. As a rule, several rows of cells are found between two neighboring capillaries. To some extent in mammalia, but especially in amphibia, single rows of cells are seen between the capillaries, so that each cell is in contact with blood capillaries, on two sides. No excretory duct can be found between the cells. The cells are polygonal in shape, 9–12 μ in diameter (smaller than the gland cells), with very finely granular protoplasm which does not stain well with eosin. The nuclei are ellipsoidal, show a fine chromatin network, and nucleoli which are never so large as those of the glandular cells.

Harris and Gow distinguish three main types of islands in the different species of animals examined : (1) Those having no distinct cells but many small deeply stained nuclei, so that the areas appear not unlike lymphoid tissue. The most characteristic islands of this type are those of the guinea-pig. (2) Those in which the cell outlines are distinct, the cells non-granular and

joined irregularly or forming a network. The areas of the armadillo, potto, and glutton are of this type. (3) Compound cell groups, in which each area is divided into smaller masses by strands of connective tissue. This type is well seen in the human pancreas and also in that of the eagle-owl. V. Ebner, however, states that he finds all these types in the same animal and thinks that the differences depend upon whether the capillaries are collapsed or distended with blood. Diamare (1899) has investigated the areas of Langerhans in teleosts, reptilia, mammalia, aves, and amphibia, finding that in all cases they consist of cords of epithelial cells separated by blood-vessels, but differing in the size and arrangement of the cell cords and in the development of the vascular rete. Pugnat, studying the pancreas of birds of many different species, finds large irregular areas consisting of small elongated cells with indistinct protoplasm and clear nucleus and regards them as lymphoid in nature. In 1904 Rennie published a most important communication concerning the areas of Langerhans in teleostei; in it he reports the results of his study of the areas in twenty-five species of osseous fishes, in all of which very large areas were found in the small islets of pancreatic tissue scattered along the abdominal vessels and among these he finds nearly constantly present a large, so-called "principal islet" a short distance in front of the spleen in the mesenteric fold between the portal vein and the mesenteric artery. Others were found almost constantly in many species. In *Lophius piscatorius*, this principal islet is very large and constant in its position and is independent of pancreatic tissue, being surrounded by a capsule of areolar tissue.

Regarding the connective tissue of the insulæ, Flint says, as a result of his investigations of the human pancreas by the Spalteholz method, that the connective tissue of the islands is definitely and characteristically arranged and sharply contrasted with that of the rest of the lobule. The islands are surrounded by a distinct fibrous capsule in relation with the alveolar framework. Septa or trabeculæ divide the area into smaller compartments containing the cells. His figure shows a septum passing through the center of the area and branches

passing off from it to either side dividing each half into compartments of fairly uniform size. Laguesse, on the other hand, states that there is no connective tissue, but a thin, homogeneous layer, which may be thickened in places ("pseudo capsule"), and that a thin, amorphous sheath accompanies the principal vessel and may spread out over the capillaries. V. Ebner says that as a rule there is no connective tissue or membrana propria between the island cells and the capillaries.

As to the blood supply of the islands, while all agree that they are richly vascularized, there is some disagreement regarding the character of the vessels. V. Ebner regarded them as venous and was supported by Diamare. Kühne and Lea state that the vessels arise partly from capillaries and partly from arterioles. Hansemann finds only capillary connections, and Opie states that "the glomerularnet work is in very free communication with the smallest arteries and that apparently the blood supply is richer than that of other parts of the lobule." Laguesse states that generally a principal vessel enters the island (rarely an arteriole, generally a large branch continuous with an arteriole). In a very recent article, which is profusely illustrated, Pensa has reported the results of vascular injections of the areas of Langerhans in a large number of species of vertebrates. He finds the areas richly vascularized, mostly by a capillary network which is a continuation of the intertubular capillary plexus of the pancreas. In some animals, as birds, guinea-pigs, and dogs, the larger areas may show, in addition to the capillary vessel, a small afferent artery, breaking up into a capillary plexus within the island, the blood being then collected into a single efferent vein. He does not state, however, how he distinguishes the arteries from the veins, and while he states that the connection is usually purely capillary, his Fig. 2 Plate IV, seems to indicate that the connection with larger vessels is very common.

By the Golgi method, Pensa was able also to demonstrate a very rich network of nerve fibers in the areas, the nerves passing along the blood-vessels and also between the cells, and being distinctly different both in number and arrangement from the nerves of the acini.

Dogiel by the Golgi method, and Kühne and Lea and also v. Ebner by injecting the ducts, showed that the areas of Langerhans are not connected by permeable ducts to the excretory ducts of the pancreas, although Lewaschew believed that some of the injection mass penetrated within the island. His results are believed to be due to an extravasation of the injection mass.

Laguesse divides the areas of Langerhans of man with respect to their size into very small, less than 100 μ. in diameter; small, 100μ to 150μ; medium, 150μ to 200μ; large, over 200μ in diameter; and giant forms (very rare), over 400μ in diameter.

After examining ten human subjects, Opie determined that there was in the head of the pancreas an average of 0.366 areas to the square millimeter, 0.36 in the body, and 0.68 in the tail, those in the tail being therefore four times as numerous in the cubic millimeter as in other parts of the pancreas. Sauerbeck in twelve human subjects finds an average of one per square millimeter, while Laguesse in six subjects finds an average of a little less than one per square millimeter and regards anything less than 0.5 per square millimeter as a diminution. He states that the one-hundredth part of the pancreas is area of Langerhans.

As to the distribution of these areas, Opie states that they are four times as numerous in the tail as in any other part of the pancreas and are situated in the center of the lobule, at least in the cat, in which he finds one area in each lobule in the splenic portion of the gland. In man, he says the number is more variable.

My own investigations on the morphology and histology of the areas of Langerhans have been made (1) by reconstruction by the Born wax-plate method of areas of Langerhans from man, cat, rabbit, and rat, and also of injected preparations from cat; (2) by careful study of serial sections of injected and uninjected preparations of pancreas of man, cat, rabbit, rat, guinea-pig, frog, and bird. The tissues were fixed as soon as possible after the death of the animal, in most cases in saturated aqueous solution of bichloride of mercury, and imbedded in paraffin, and serial sections averaging 5μ in thickness were cut. These were stained in hæmatoxylin and eosin, Mallory's connective tissue

stain, Weigert's elastic stain, and in some few cases in Heidenhain's iron-lac hæmatoxylin and in saffranin and *Licht-grün*. Some of the most satisfactory and instructive preparations were those in which there was some congestion so that there was a physiologic vascular injection. These were often more satisfactory for study of the structure than those injected with a gelatin mass, in which the cells of the areas were often more or less changed. For the injected preparations, the simple Berlin blue and carmine gelatin masses were used and also a double injection, the veins being filled with the Berlin blue or carmine gelatin, while the arteries were filled with a granular mass (cinnabar or ultra marine blue.) In this procedure it was difficult to prevent a rupture of the smaller vessels and an extravasation of the granular mass, but some areas of the pancreas often showed a good double injection. In the estimation of the size of the areas, sections near the center of the areas were measured for the first two dimensions, while the third was obtained by counting the number of sections of the series through which the given area extended and multiplying by 5 μ, the thickness of the sections. It is easy in nearly all forms to distinguish the central from the peripheral sections, not only by their size but also by the fact that in the center of each area the smallest cords of cells and the largest blood-vessels are found. The volume, as given, is never exact, since it is obtained by simply multiplying the three dimensions, together. However, it answers the purposes of comparision and any more exact method would be exceedingly difficult because of the irregular shapes of the areas.

In the *guinea-pig*, I found an average of 1.07 areas per square millimeter or 1.14 areas per cubic millimeter. As the average volume of these areas was .0091 cu. mm., about one hundredth of the pancreas consists of island tissue. The areas vary greatly in size and shape as may be seen from a comparison of the measurements of the following ten areas·

1.	0.40 x 0.32 x 0.285 mm.	0.036 c. mm.
2.	0.13 x 0.30 x 0.120 mm.	0.0047 c. mm.
3.	0.29 x 0.33 x 0.115 mm.	0.01 c. mm.
4.	0.14 x 0.45 x 0.185 mm.	0.012 c. mm.
5.	0.15 x 0,32 x 0-175 mm.	0,0098 c. mm.

6.	0.13 x 0.18 x 0.11	mm.....	0.0026 c. mm.
7.	0.10 x 0.13 x 0.125	mm.....	0.0016 c. mm.
8.	0.12 x 0.17 x 0.18	mm..... ..	0.0037 c. mm.
9.	0.15 x 0.2 x 0.25	mm.....	0.0065 c. mm.
10.	0.12 x 0.27 x 0.28	mm.....	0.0098 c. mm.

They are usually oval or elongated in shape with quite regular contour, rarely lobulated or even with marked projections. Near the periphery of the pancreas, the areas are relatively small and spherical or oval, while near the central portion of the gland in the neighborhood of the larger ducts, especially at about the junction of the splenic and middle thirds, the areas are large and prominent. In this region, especially large islands are occasionally found, analogous to the giant areas described by Laguesse in the human pancreas. These often seem to have no connection with the pancreatic acini, being surrounded by the fibrous connective tissue and adipose tissue around the duct. While they vary in shape, many of them are much enlongated, being several times as long as they are wide, some having been found measuring 0.9 to 1.0 millimeter in actual length and 115 to 120 μ in width. In this region also masses of lymphoid tissue are occasionally seen, easily differentiated from the islands by the different character of the cells and by the arrangement of the cells and blood-vessels. The number, position, and relations of these central areas are represented in Fig. 1, a low power drawing of one of the main ducts of the pancreas of a guinea-pig, with the surrounding connective tissue and blood-vessels. In this small mass of tissue, measuring 3.2 mm. in length, five relatively large areas are seen, and three others were just beyond the field of vision. A small amount of pancreatic tissue is seen in the section, but it has no close relation to the areas of Langerhans, which are distinctly encapsulated. Occasionally at the periphery of the pancreas in the adipose tissue of the surrounding mesentery, I have seen areas which appeared to be free. Usually by tracing such areas through the series of sections in which they occur, some few pancreatic tubules are seen in loose or close relation with them.

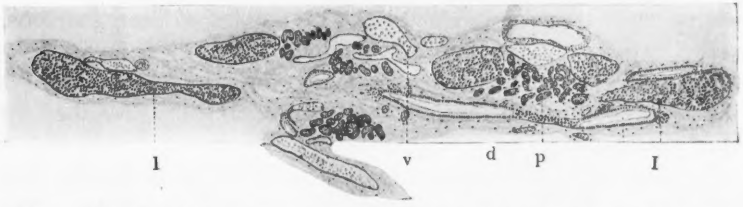


Fig. 1.—Large duct from pancreas of guinea-pig, showing surrounding connective tissue and blood-vessels with a small amount of glandular tissue and five relatively large areas of Langerhans. I, areas of Langerhans; p, pancreatic acini; v, blood-vessels; d, duct. × 25.

The discovery of such relatively isolated islands suggested to me the possibility that isolated areas might be found in the

mesentery of some guinea-pigs, as in the teleostei, as shown by Rennie. Since the observation was made, however, only a small number of guinea-pigs have been at my disposal, and in these I have been unable to find isolated areas at any considerable distance from the pancreas, although, as in the teleostei, strands of island-containing pancreatic tissue accompany the vessels for some distance. With the exception of the relatively isolated areas above described, the insulæ of the guinea-pig's pancreas are in close relation to the pancreatic acini, being separated from them only by the membrana propria, which is often difficult to demonstrate, and sometimes for a distance by a capillary.

The cells are usually described as of two kinds, but to my mind, this classification scarcely covers all the cells found in the islands. (1) The great majority of the cells are, as has often been stated, polyhedral in shape, have homogeneous or nearly homogeneous protoplasm staining faintly with eosin, and have spherical or oval nuclei which are about the size of the nuclei of the acinal cells and show chromatin granules and one or two nucleoli. Many of the cells show distinct cell outlines. (2) There are a few cells with homogeneous protoplasm and very large faintly staining nuclei. (3) Small cells, with small, deeply staining nuclei occur. (4) Large cells with eosinophile granules in the protoplasm and with large, faintly staining nuclei are found. (5) Eosinophile cells with small, excentric, deeply staining nuclei are usually not very numerous and may be found either near the periphery of the island or in the central portion adjoining a blood-vessel. In well-fixed and well-stained sections, I have never found the syncytial appearance described by Hansemann or the masses of nuclei without cell differentiation described by Harris and Gow.

In every respect the structure of the central insulæ surrounding the duct is the same as that of the intralobular areas. The insular cells are arranged in irregular, sinuous bands, sometimes, especially in the central part, consisting of a single row of columnar cells with deeply staining and often quite large nuclei, sometimes of a double row of such cells, while in places, especially at the periphery of the area, the cells are grouped into small masses

The bands of cells follow the arrangement of the blood-vessels, but I failed to find any arrangement of blood-vessels or cords of cells which I could regard as characteristic for the species. In some sections, a larger vessel may be seen entering at one end of the area and passing through the center of the island, dividing it into two more or less equal portions; smaller branches passing off from the sides of this main vessel subdivide each half into larger or smaller compartments, giving a figure not unlike the figure and description given by Flint of the arrangement of the connective tissue in the human areas. Very little or no connective tissue, however, accompanies the vessels. In other sections, the vessel enters at one side soon dividing into numerous larger branches, which pass obliquely through the area in every direction, giving off many secondary branches which frequently anastomose by means of true capillaries.

The capsule, if such it may be called, is very delicate, and the walls of the insular capillaries seem to rest directly on the epithelial cords. When some shrinkage has taken place, however, the capillary occupies the center of a space which separates it from the epithelial cords, and in preparations well stained with Mallory's connective-tissue stain, delicate sheaths of connective tissue are seen to surround the blood-vessels; delicate lines of blue outline the cells, while a very thin capsule of connective tissue separates the area from the pancreatic acini.

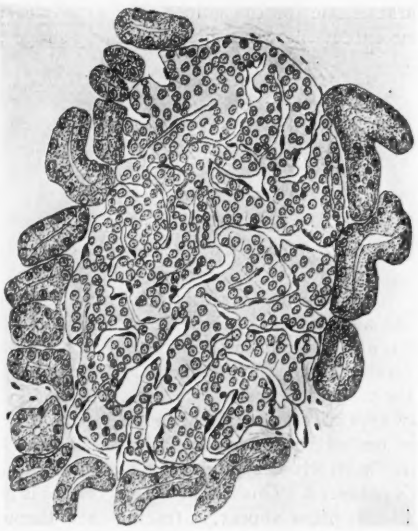


Fig. 2.—Area of Langerhans from pancreas of guinea-pig. Section through center of area. ×20.

The amount of connective tissue in the areas is so much less than

that of the surrounding acini, and the arrangement is so different that the areas are sharply differentiated from the acini by the arrangement of the connective tissue alone. Fig. 2 shows a section through the center of a rather typical intralobular area of Langerhans of the guinea-pig. It is surrounded by pancreatic tubules except at one end, where the area reaches the periphery of the lobule and is limited by the interlobular connective tissue. The large columnar cells, single or double rows of which form the central cords of the area, and the more crowded cell masses at the periphery are well seen. These peripheral masses resemble somewhat the description given by Harris and Gow of the areas of Langerhans of the guinea-pig,—masses of nuclei, not differentiated into cells, and somewhat resembling lymphoid tissue—but the area as a whole is distinctly different.

In the *rat* also, the areas near the center of the pancreas are larger and more numerous than those near the periphery, but I have never, in the material at hand, been able to find the large isolated or nearly isolated central areas so characteristic for the guinea-pig. The measurements given below indicate to some extent the variations in size and shape of the areas of Langerhans in the rat.

1. 0.53 x 0.27 x 0.26 mm. .....0.037 c. mm.
2. 0.50 x 0.43 x 0.32 mm. .....0.0688 c. mm.
3. 0.14 x 0.38 x 0.22 mm. .....0.018 c. mm.
4. 0.22 x 0.23 x 0.40 mm. .....0.02 c. mm.
5. 0.26 x 0.62 x 0.475 mm .....0.076 c. mm.
6. 0.16 x 0.25 x 0.30 mm. .....0.012 c. mm.
7. 0.30 x 0.60 x 0.51 mm. .....0.092 c. mm
8. 0.14 x 0.18 x 0.29 mm. .....0.007 c. mm.
9. 0.17 x 0.51 x 0.465 mm. .....0.04 c. mm.
10. 0.15 x 0.18 x 0.21 mm. .....0.0376 c. mm.

The average size of these ten areas is .0376 c. mm. and, as the average number is 0.7 per mm. in the material at hand, about $\frac{3}{100}$ of the pancreas consists of insular tissue. It is somewhat difficult to be sure of counting areas correctly in the rat, since most of the areas are irregular and lobulated so that often areas may appear as two quite widely separate areas in one section, while in another they unite into one relatively large island. This can easily be seen from Plate XII, Fig. 1, which reproduces a model of a typical area of Langerhans from the rat's pancreas. This island of Langerhans is not nearly so large nor so lobulated as islands often appear, in tracing them through the series of sections in which they occur. It consists of two distinct lobules of insular tissue, well separated at the periphery, but joined at the center into a solid mass. The sponge-like appearance of these areas is well seen in the figure, since, as stated before, the relatively loose, band-like arrangement of the cells at the center is concealed to some extent by the more solid masses of small cells at the periphery, through

which only the small capillaries and the few afferent and efferent vessels pass. In the rat, however, the cells at the periphery are to some extent arranged in irregular cylinders resembling tubules in contour, but having no lumen and having a very free communication with other cylinders. The cells of the rat's insulæ are very similar to those already described for the guinea-pig. Large columnar cells with large nuclei arranged in definite bands consisting of one to two rows of cells occupy the central portion, while at the periphery most of the cells are smaller, the nuclei more crowded and the arrangement less band-like; the blood-vessels are smaller and less prominent. Fig. 3 represents the appearance of a section through the central part of a small area of Langerhans from the rat's pancreas.

Fig. 3.—Section through central part of area of Langerhans from pancreas of rat. × 200.

In this series of sections, there was passive congestion, so that all veins and venous connections were filled with blood, while the arteries were as a rule empty. It was therefore an especially favorable series of sections for determining the relation of the vascular supply to the pancreatic vessels. As stated before,

v. Ebner and Diamare regarded the vessels of the areas as venous, while Laguesse, Opie, Kühne and Lea, Pensa and others believed it to be arterial, and Hansemann finds only capillary connections. After a most careful investigation of this series of sections as well as of many others from this and many other species, I have never been able to demonstrate an arterial connection with the vessels of the areas, since I have not found a vessel with distinct muscular coat either entering the areas or sending branches into them. Sometimes an arteriole was seen apparently approaching an area, but on tracing it through the series of sections, it was seen to turn aside or pass over the area without communicating with it. In cases of venous congestion, the blood-vessels of the islands are always packed with blood, and in a few cases, as in Fig. 4, I have been able to trace the main vessel, which have regarded as afferent, directly to a vein, beside which ran an artery with distinct muscular coat. In double injections, as will be shown later, it is always the venous injection mass which fills the blood-vessels of the areas. I have therefore regarded the blood-vessels of the areas as venous with abundant capillary connections with the surrounding interacinar capillary plexus.

Nearly always in tracing areas through a series of sections, at least one and sometimes more than one larger vein, usually arising either from an interlobular or intralobular vein, has been seen to enter the area and this I have designated as the principal or afferent vein, while several smaller venules and large numbers of capillaries leave the area in all directions. Since all of these vessels have the same structure, it is difficult, except by the size, to determine which are afferent and which efferent, and we can say only that several larger veins and numerous capillaries form a rich plexus of large, irregular, thin-walled blood-vessels, which, in accordance with Minot's characterization, I regard as sinusoids. The insular vessels always have a different arrangement from that of the interacinar vessels, but the arrangement is variable, especially in sections. Since the areas of the rat are usually much longer than they are wide, the principal vessel usually enters at the side, often passing, as in Fig. 4, to near the center of the island and there breaking up into branches,

which divide and redivide, the secondary branches often communicating by means of capillaries. Since Fig. 4 represents a section only 5μ in thickness, some idea of the rapidity of the branching and the centralization of the larger branches may be gained, although a thicker section would show greater continuity of the vessels. In sections stained with hæmatoxylin and eosin, the endothelial walls of the blood-vessels seem to rest directly upon the cords of epithelial cells constituting the island, and in most places no connective-tissue capsule can be seen. Where the area reaches the periphery of the lobule as seen in Fig. 3 and as is quite common, especially with the larger areas in the central portion of the pancreas, loose connective tissue forms its boundary and occasionally nucleated connective tissue may be seen in other places separating the area from the acini. In sections stained with Mallory's connective-tissue stain, a delicate blue line is seen separating most of the cells; a thin, but nearly if not quite complete, capsule of connective tissue surrounds the area and sends in still more delicate sheaths for the blood-vessels.

Fig. 4.—Section of area of Langerhans from pancreas of rat in which the veins were filled with blood. The figure shows the connection with the large vein and the arrangement of the sinusoids within the island as seen in a thin section. ×200.

In the *rabbit*, I have found on an average about one area for each square millimeter of section. As the average size of areas measured was .0034 c. mm., about $\frac{3}{1000}$ of the pancreas was insular tissue. The measurements taken were as follows·

1.	0.12 x 0.22 x 0.09 mm	0.0024 c. mm.
2.	0.15 x 0.23 x 0.15 mm	0.0052 c. mm.
3.	0.08 x 0.23 x 0.18 mm	0.0033 c. mm.
4.	0.12 x 0.18 x 0.10 mm	0.0021 c. mm.
5.	0.16 x 0.20 x 0.15 mm	0.0048 c. mm.
6.	0.13 x 0.16 x 0.18 mm	0.0037 c. mm.
7.	0.15 x 0.23 x 0.08 mm	0.0028 c. mm.
8.	0.10 x 0.11 x 0.095 mm	0.0011 c. mm.
9.	0.14 x 0.18 x 0.255 mm	0.0064 c. mm.
10.	0.15 x 0.15 x 0.10 mm	0.0023 c. mm.

A comparison of these measurements shows a considerable difference in the actual size of the areas, since Number 9 is nearly six times as large as Number 8. As may be seen from the measurements, as well as from Plate XII, Figs. 2 and 3, which reproduce two wax reconstructions of fairly typical islands from the pancreas of a rabbit, the islands are nearly always considerably longer in one dimension than in the other two. They are not, however, at all regularly oval, since there are numerous projections which run out between the pancreatic acini. The cells are of about the same types as described for the guinea-pig and rat, but in the sections observed by me there was an unusually large number of the large distinctly contoured eosinophile cells, with large, excentric, deeply staining nuclei. Such cells are more numerous near the periphery of the area. These often seem to merge into the acinal cells so gradually that no line of demarcation can be made out. In some places the lines of nuclei in the bands of cells pass out regularly into the cells of a longitudinally cut tubule which shows the typical basal position of the nuclei and the nuclear stain of the basal protoplasm, eosinophile granules being present in the central portion of the protoplasm. The same or similar appearances are not rarely seen in all forms in which the capsule is very thin and the relation to the acini very close. I have, however, regarded the two structures as separate unless, as frequently happens in the rabbit, the connection can be clearly traced through several sections of the series. This has been done in numerous instances in the rabbit and the connection may be seen in the models represented in Plate XII, Figs. 2 and 3, and also in the section shown in Fig. 5. This appearance can best be explained by the fact shown by Pearce and others that the areas develop from the same epithelial anlage as do the pancreatic tubules, and it may easily be

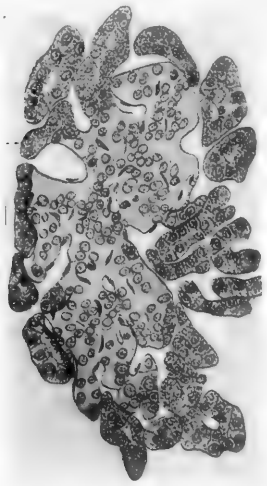


Fig. 5.—Section through the center of an area of Langerhans from pancreas of the rabbit; p, connection with pancreatic tubule. × 200.

that in the rabbit and other lower forms, as well as in some pathologic conditions in the human infant, the connection with the tubules remains unbroken. As seen in Fig. 5, the band-like arrangement of the cells is much less marked in the rabbit than in other species, the bands usually consisting of so many and so irregularly arranged cells that they may be spoken of rather as cell masses.

The character and arrangement of the blood-vessels and connective tissue are very similar to that already described for the guinea-pig and rat. Fig. 6 represents a curious anomaly, two examples of which were found in the sections of the rabbit's pancreas studied by me. These were not reconstructed, but were carefully traced through the entire series of sections in which the area appeared. They were nearly spherical masses of perfectly normal island tissue, which however formed merely a shell, the interior being filled with blood and having an endothelial lining. The occurrence of this blood vesicle or sinus, although only as an anomaly, may serve to lend some support to the idea that the circulation here is sinusoidal. Added interest is given to the anomaly by the fact that Laguesse describes in sheep embryos forms in which the island cells form hollow balls, the interior being filled with blood cells. A similar appearance (blood vesicle) in the petromyzon is mentioned by Pensa.

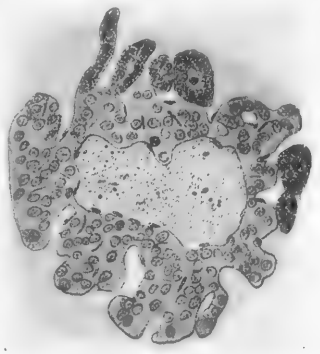


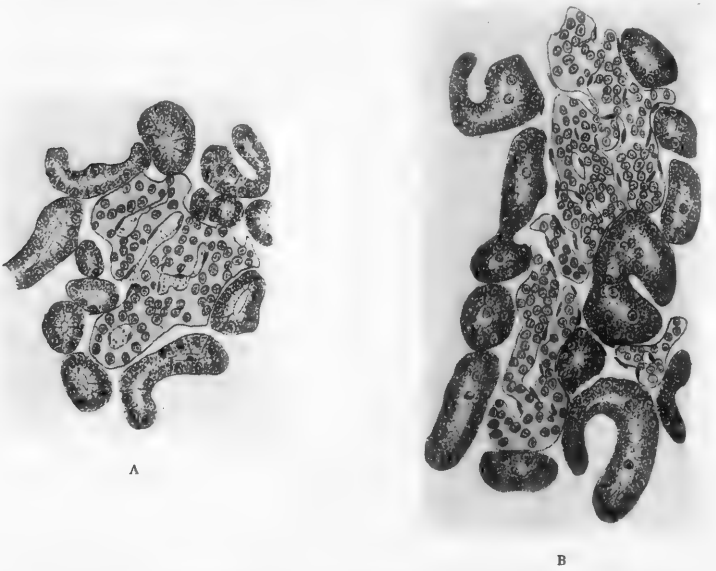
Fig. 6.—Section through center of a vesicular area from pancreas of the rabbit, the interior of the area being filled by a large blood sinus surrounded by endothelial cells which rest directly on a shell of normal island tissue. +200.

In the *cat*, the areas are as a rule much smaller than in the other forms studied. Twenty areas were measured, taken from different portions of the pancreas and from two different animals. The average size of all the areas was about 0.0027 c. mm. The average number per mm. was 1.08, so that about $\frac{8}{1000}$ of the pancreas consisted of island tissue.

	Cat I		Cat II	
1.	.08 x .105 x .18 mm.	.0015 c. mm.	12 x .16 x .11 mm.	.0021 c. mm
2.	.14 x .19 x .125 mm.	.0033 c. mm.	.11 x .17 x .15 mm.	.0028 c. mm.
3.	.12 x .25 x .10 mm.	.003 c. mm.	.19 x .32 x .135 mm.	.0082 c. mm
4.	08 x .17 x .09 mm.	.0012 c. mm.	.07 x ,20 x .13 mm.	.0018 c. mm.
5.	.09 x .18 x .14 mm.	.0023 c. mm.	.12 x .19 x .20 mm.	.0046 c. mm.
6.	.11 x .13 x .13 mm.	.0019 c. mm.	.13 x .25 x .125 mm.	.0041 c. mm.
7.	.08 x .14 x .14 mm.	.0016 c. mm.	.11 x .20 x .135 mm.	.003 c. mm.
8.	.11 x .18 x .11 mm.	.0022 c. mm.	.14 x .17 x .12 mm.	.0029 c. mm.
9.	.14 x .16 x .20 mm.	.0045 c. mm.	.20 x .22 x .125 mm.	.0024 c. mm.
10.	.10 x .14 x .15 mm.	.0021 c. mm.	.12 x .17 x .12 mm.	.0028 c. mm.
	Average size	.0024 c. mm.	Average size	.0034 c. mm.

It will be seen that the average size in the second series is greater

than in the first and that the areas are generally more nearly spherical or oval than in certain of the other mammals observed, though many of them are considerably elongated, as seen in Fig. 7, B. In Plate XIII, Figs. 1 and 2 are reproduced wax reconstructions of two small areas of Langerhans from the pancreas of an adult cat. These appear, as a rule, unless the sinuses are distended with blood or injected, as nearly solid masses of cells, most of the capillaries being entirely collapsed and seen only as a line of endothelial cells. Especially on the surface are the vascular openings small, so that the cellular elements much predominate. The varieties of cells are in large part the same as those already described; the cells are arranged in irregular masses and to some extent in bands consisting of a single or double row of cells. While the arrangement is variable and all possible forms are seen, it is not unusual to see the cords with the intervening sinusoids passing entirely or nearly across the area, as seen to some extent in both A and B of Fig. 7.



A B

Fig. 7, A and B. Central sections of two areas of Langerhans from pancreas of cat. × 200.

A number of double injections of the pancreas of the cat were attempted and while the results were not so successful as could be desired, in certain portions of the tissue the injection was such that arteries containing the red granular mass could be found, by the side of which could be traced a vein containing the blue gelatin mass and from there could be traced the principal branches passing to a Langerhans area. Certain of the sections from this tissue were cut relatively thick, so that one section would often contain the whole or nearly the whole of some of the smaller areas, and thus it was possible

to get in sections a very good idea of the arrangement of the sinusoids and capillaries in the islands. In Plate XIII, Figs. 3, 4, 5, and 6, are reproduced models reconstructed from thin sections of these injected areas, the blood-vessels only being used in the reconstruction. Three main types of arrangement of vessels may be noted —(1) The vessel runs along the side, either outside or just within the margin, of the area and sends branches mainly from one side into the area; the branches cross the area, branching and winding through it in every direction, and being often connected by anastomosing capillaries. (2) One or two main branches pass through the center sending branches in every direction. (3) The main branch reaches the center of the area and there breaks up into branches which radiate in every direction.

In the models, the arrangement can be quite easily traced in the smaller islands of Langerhans, but in the larger, the arrangement becomes so complicated that tracing is difficult, and the figure can give only a very incomplete idea of the arrangement. In the small islands, there is usually one main (probably afferent) vein, which passes to one of the larger veins, either interlobular or intralobular. Plate XIII, Fig. 3, shows a small and simple area in which the arrangement of the blood-vessels may be easily traced. A relatively large vein runs along the side of the island and sends off a short principal afferent vein. This breaks up almost at once into four smaller vessels, three of which may be seen in the figure. After winding through the area and branching and anastomosing several times, five vessels leave the area, two being capillary in size and the others larger. In Plate XIII, Fig. 4, the main afferent vessel divides, soon after entering the island, into five main branches which branch and anastomose many times, the blood leaving the area by one large efferent vein and six capillaries. The island represented in Plate XIII, Fig. 5, is composed of a plexus consisting of the branchings and anastomoses of two larger vessels and twelve capillaries, while the still larger area represented in Plate XIII, Fig. 6, shows a plexus composed of four larger vessels and fifteen capillaries with their branches. Two of the large vessels form an S-shaped curve and again meet to form the core of the area, the other branches and capillaries twisting around and between them to make up the intricate vascular plexus seen in the figure.

The *human areas of Langerhans* seem much more variable in structure than those found in the other species studied, age and the general condition of the body being more important factors in their structure than appears to be the case in most of the other vertebrates. This is especially true as concerns the insular connective tissue. I have counted and measured areas of Langerhans (1) from a four-year-old child (possibly syphilitic), (2) from a new-born, normal, healthy infant, and (3) from an apparently normal human adult. The measurements are as follows:

	I		II	
1.	.21 x .22 x .28 mm.	.0129 c. mm.	.15 x .17 x .13 mm.	.0033 c. mm.
2.	.20 x .28 x .35 mm.	.0196 c. mm.	.20 x .21 x .18 mm.	.0076 c. mm.
3.	.23 x .26 x .30 mm.	.0179 c. mm.	.16 x .18 x .20 mm.	.0058 c. mm.
4.	.16 x .25 x .20 mm.	.008 c. mm.	.15 x .15 x .17 mm.	.0038 c. mm.
5.	.15 x .17 x .23 mm.	.0059 c. mm.	.14 x .15 x .18 mm.	.0037 c. mm.
6.	.23 x .28 x .42 mm.	.027 c. mm.	.13 x .15 x .14 mm.	.0027 c. mm.

7.	.16	x	.225	x	.27	mm.	.0097	c. mm.	.16	x	.19	x	.21 mm.	.0064 c. mm.
8.	.19	x	.20	x	.265	mm.	.01	c. mm.	.12	x	.14	x	.17 mm.	.0029 c. mm.
9.	.18	x	.22	x	.225	mm.	.0089	c. mm.	.17	x	.23	x	.15 mm.	.0059 c. mm.
10.	.24	x	.28	x	.29	mm.	.0194	c. mm.	.12	x	.21	x	.17 mm.	.0043 c. mm.
	Average size						.0139	c. mm.	Average size					.0047 c. mm.

III

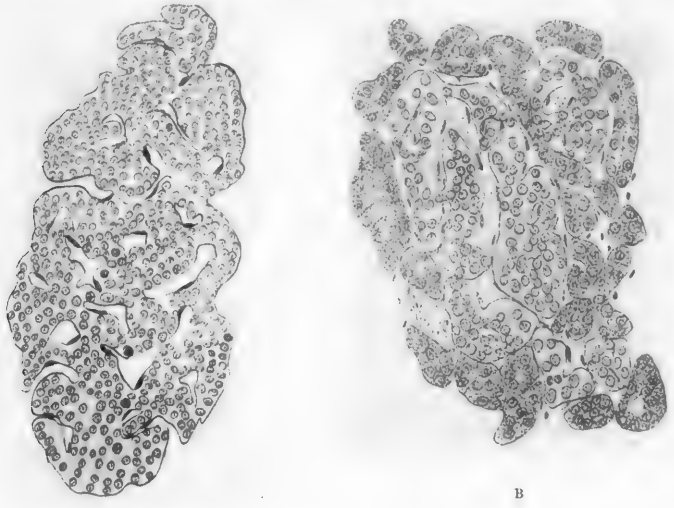
1.	.25	x	.35	x	.325	mm.	.0284 c. mm.
2.	.23	x	.24	x	.225	mm.	.0124 c. mm.
3.	.20	x	.38	x	.225	mm.	.0171 c. mm.
4.	.23	x	.33	x	.225	mm.	.017 c. mm.
5.	.18	x	.20	x	.16	mm.	.0057 c. mm
6.	.24	x	.25	x	.215	mm.	.0129 c. mm.
7.	.24	x	.30	x	.32	mm.	.023 c. mm.
8.	.17	x	.23	x	.275	mm.	.0108 c. mm.
9.	.23	x	.34	x	.25	mm.	.0195 c. mm.
10.	.15	x	.20	x	.215	mm.	.0065 c. mm.
	Average size						0.0153 c. mm.

In Series I of the table, the number of areas averaged $3\frac{1}{7}$ to each c. mm. of the section, so that about $\frac{4}{100}$ of these sections was area tissue. In Series II, the average number was only 1.3 to each c. mm. of the section and hence only about $\frac{8}{1000}$ of this pancreas was island tissue. The average number of areas in each sq.mm. of the adult pancreas (Series III) is 1.5, making about $\frac{8}{100}$ of the pancreas consist of insular tissue. My figures are slightly different from those given by Opie, Laguesse, and others, since Laguesse finds that on the average only $\frac{1}{100}$ of the human pancreas is island tissue. This can be explained only as an individual variation, since in the figures of the observers mentioned there is quite as marked a variation between any two cases. It is worth noting that the average size in the adult is actually greater than in either of the other cases, while in the four-year-old child it is greater than in the newborn infant. This is quite different from the results frequently reported, since it is usually stated that both the size and the number of islands of Langerhans diminish with age, being greatest in the fœtus and new-born. In the four-year-old child, however, the number in a sq. mm. was greater than in either of the others, and the proportion of area to glandular tissue was much greater in this than in either of the others.

The shape of these areas varies greatly, but is in the main ovoid, though the contour is quite irregular. Something of the shape and general appearance may be seen in Plate XIV, Figs. 1 and 2. Fig. 1 reproduces a wax reconstruction of a fairly typical area from the adult human pancreas, while Fig. 2 is the negative of Fig. I, representing the strands of connective tissue with blood-vessels, etc. Since there was some shrinkage of the epithelial cells, the strands are relatively somewhat larger than they should be, but otherwise indicate the arrangement of the blood-vessels in the larger and more complicated areas like the human. Plate XV, Figs. 1 and 2, reproduce the interior view of the models represented in Plate XIV. The looser and more open appearance of the central portion of the areas is readily seen in these figures. It may also be seen that in some regions two and in others three larger sinusoids run through the area in

either direction, so that we find much the same appearance in whatever direction the sections are cut. These large sinusoids are connected at intervals by smaller vessels and, as the greater part of the island is composed of superposed layers of similar arrangement, it may easily be seen why so large a number of sections through the larger portion of the area show a very similar arrangement of cords of cells and blood-vessels. The rich branching of the larger vessels and the connections of the smaller ones may also be seen in the figure.

Since the arrangement of the blood-vessels regulates the arrangement of the bands and groups of cells, a description of the one figure necessarily involves a description of the other. The resemblance of the cords of cells to tubules and acini appears more marked in the interior of the model than in the exterior view. It may be repeated, however, that not only is no lumen found, but there is not even any arrangement of cells which would suggest a lumen. The cells are most irregularly grouped, and the only suggestion of regularity of arrangement seems to be due to their following the arrangement of the blood-vessels. The cells of the human areas vary in size and structure and may be divided into about the same classes as were described for the guinea-pig. The amount and arrangement of the insular connective tissue vary more than any other portion of the structure. In the child's pancreas, there is very little or no connective tissue within the island, the sinusoids resting directly upon the epithelial cells. A space may often be seen between the capillary wall and the



A B

Fig. 8, A and B.—Sections of areas of Langerhans from pancreas of four-year-old child. × 200.

epithelial cells, indicating that the cells have shrunken somewhat either in the fixation or as a result of post-mortem change. No such spaces were observed

in the fresh pancreas of the new-born infant. No connective tissue can be made out in the pancreas of the four-year-old child with the ordinary stains, but with Mallory's stain a delicate capsule surrounding the area and delicate sheaths around the blood sinusoids can be made out.

In the pancreas of the new-born infant, nearly all the areas observed were found in the interlobular connective tissue and surrounded by it, but no connective tissue could be made out within the areas. In Fig. 8 are reproduced sections of two insulæ from the pancreas of the four-year-old child. The cells are somewhat smaller, the nuclei appearing smaller and more crowded than in the islands of the adult pancreas. This is still more the case in the areas of the younger child. In Fig. 8, B shows an area which has irregular projections into the acinal tissue, as if it had been somewhat broken up by the ingrowth of acini. It is evidently not so near the center of the island as A, which represents a very typical section. In the adult, at least in most of the islands seen by me, there is more connective tissue, forming rather definite trabeculæ, which divide the island into smaller compartments containing the cells; this recalls the description given by Harris and Gow, who, because of this tendency, speak of the human areas of Langerhans as compound. Sometimes the connective tissue forms, with the larger blood-vessels, one or many larger trabeculæ passing through the center, other septa passing to the sides, much as described and figured by Flint. Such an area is shown in Fig. 9, A, showing the central strand cut longitudinally, while in B, it appears to be cut transversely, giving the radiating

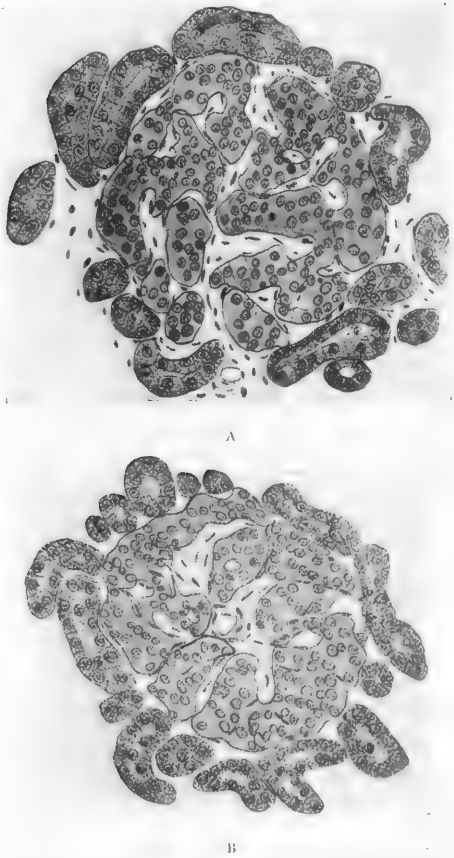


Fig. 9, A and B.—Central sections through two areas of Langerhans of the human pancreas. × 200.

arrangement of the secondary strands, similar to that described for the blood-vessels of the bird. Such differences may be readily understood from a reference to Plate XV, Fig. 2, if we imagine a section cut through the large sinusoids which run nearly parallel to the plane of the section in that figure, we will have much

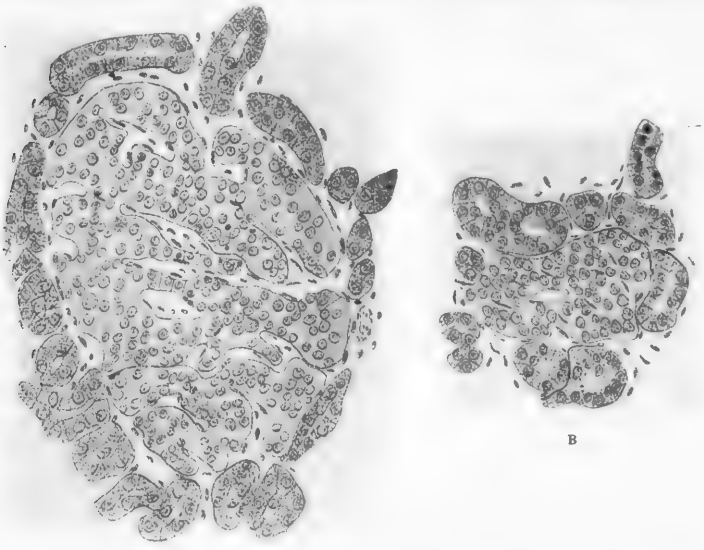


Fig. 10, A and B.—Sections of areas of Langerhans from pancreas of human adult. A, near center of area; B, near periphery. × 200.

the appearance given in Fig. 9, A. If the section is cut in the same direction, but between two layers of the larger sinusoids, we will get more the appearance given in Fig. 9, B. In general, however, the strands of connective tissue in the human areas, like the sinusoids of other forms, have a very irregular arrangement, scarcely any two islands presenting exactly the same appearance.

A rather definite capsule of nucleated connective tissue surrounds most of the areas. The only thing which is constantly noted, in the child as well as in the adult, is that in the interior of the islands the strands of connective tissue as well as the blood-vessels contained in them are larger and the masses of cells smaller and more band-like, while the individual cells are generally larger than at the periphery. This makes it easy, regardless of the size of the section, to distinguish a central from a peripheral section. Fig. 10 may serve to illustrate this point, A, of this figure, as well as A and B of Fig. 9; representing sections from near the center of an area, while Fig. 10, B, represents a section near the periphery. The increase of connective tissue with age or with general sclerosis of the gland and the very small amount in younger individuals and in animals seem to indicate that the formation of the connective tissue within the islands is secondary.

In birds, the areas of Langerhans have been found by Mouret, Harris and Gow, Pugnát, Diamare, and others. Diamare asserts that after careful study of numerous serial sections, he is able to affirm that this structure in birds is entirely similar to that in other vertebrates—"the islands consist of very vascular epithelial cords." Pugnát studied the pancreas in many species of birds, but described it especially in the dove. He states that the pancreas consists of three lobes, each with a distinct duct and that the cells do not form acini, but rather a reticular structure like the liver. The islands, he states, are large and irregular, consisting of small, elongated cells with indistinct protoplasm and faintly staining nuclei. Both Mouret and Pugnát regard them as lymphoid structures. I have examined the areas of Langerhans in the dove and in the goose and found them similar, but because of the greater distinctness of the islands in my sections from the dove, my descriptions and figures will be taken from this animal.

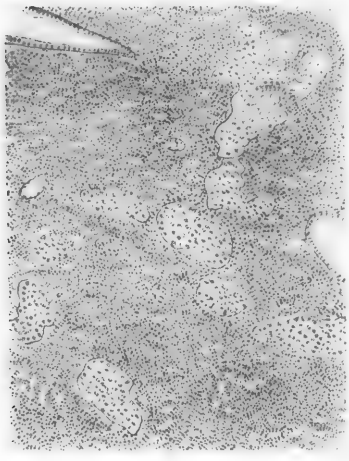


Fig. 11.—Portion of section through central part of dorsal pancreas of dove. × 50.

In the *dove* the two main lobes of the pancreas are found n the loop of the duodenum, one ventral and one dorsal and separated by the mesentery. In the ventral lobe, the areas of Langerhans are few, small and distinct. In the dorsal lobe, on the other hand, they are large and prominent, and it is from this lobe that my figures are taken. The pancreas was congested and all the blood-vessels of the areas were filled with blood and hence were very distinct. It is difficult, on account of the diffuse character of these areas, to determine with any exactness their size or number. They are larger and more numerous near the central part of the lobe than at the periphery. Fig. 11, a low-power drawing of a small portion of this part of the dorsal pancreas, may serve to show the size and irregularity of shape and apparent number of the islands in this region.

Several of these, however, unite into one area in a later section, so that the

number is smaller and the size greater than is apparent in any one section. The lighter areas represent the islands of Langerhans, and the darker portion the glandular t ssue, which I may say in passing seems to me to form definite tubules and acini, as in the pancreas of other vertebrates and not, as stated by Pugnát, a recticular stucture like the liver, although under low power it does present a reticular appearance.

Fig. 12.—Section through central portion of dorsal pancreas of dove. × 200.

The cells of the bird's islands are smaller and more uniform in size and structure than those found in the islands of any other vertebrate examined, scarcely any variation in size or stain of nuclei being noted. The cell outlines can scarcely be made out. The nuclei are small, round, or more often oval, with a very distinct nucleolus. Both nucleus and protoplasm stain faintly. The cells are arranged in definite cords between the sinusoids, which here are wide and prominent because of the congestion. Fig. 12 reproduces a central section of an area of Langerhans from the pancreas of the dove, the blood cells in the sinusoids being omitted. Fig. 13 reproduces three areas of Langerhans from the pancreas of the dove, in which the blood-filled sinusoids are especially emphasized, the cells appearing lighter in the figure. They show three types of arrangement: A shows the origin from a larger vein of the main afferent vessel, which enters the island, passing along one side and dividing into two main branches near the end. Most of the other branches are given off from one side of the main sinusoid, these again dividing and passing in different directions. A large branch is given off from the main sinusoid, which passes back, winding partly under the latter, and supplies the proximal portion of the area This is

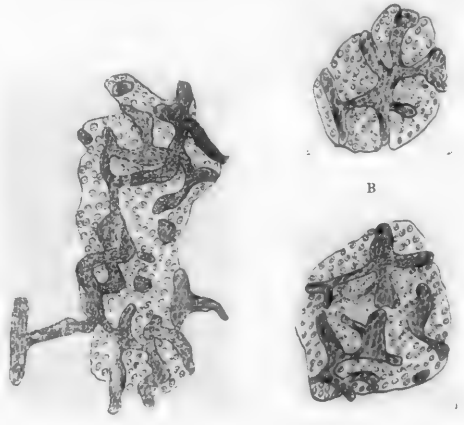


Fig. 13, A, B, C.—Three sections of physiologically injected areas of Langerhans of dove, the injected sinusoids being especially emphasized in the figure. × 200.

a relatively thin section, so that many of the tortuous capillaries are discontinuous, but it indicates very well the connections and arrangement of the insular vessels. B shows the main sinusoid passing through the center of the area and sending branches to either side, while C represents a radial branching from a central large sinusoid. C might, as will be readily seen, represent a transverse section of B.

In the frog, the islands stain more deeply and are so diffusely and irregularly scattered among the pancreatic tubules that counting and measuring are very difficult. It is almost impossible to determine how many of the small island masses seen among the acini belong to a single area or to be certain as to the limits of the areas. The most of the area sections seen by me were relatively small, averaging .002 c. mm. in size, the dimensions averaging about 90 μ x 120 μ x 210 μ. The cells differ somewhat in character from those in the other areas studied, since very tall columnar cells with elongated, deeply stained nuclei predominate. These are crowded together, usually in a single row, especially in the central portion to form the very distinct cell bands so characteristic for amphibia; they resemble those described and figured by Diamare for the triton. He states that "the small epithelial cords are rather delicate, formed of rather slender cells, closely approximated and are separated by large capillaries. These last evidently correspond to the 'large venous sinuses' of v. Ebner. No capsule separates the areas from the zymogenic tissue." Pensa also in speaking of the cylindric cells of the islands of amphibia, says: "These cells are not aggregated into cords but are arranged in linear series, one after the other in single file." In addition to these narrow crowded cells which make up the central cords, masses of polygonal cells with spherical nuclei are usually found, especially at the periphery of the islands. V. Ebner described these islands in the frog as early as 1872, stating only that they showed no lumen penetrable by a mass injected into the duct and that rather large veins are found near the islands. In his later communication in 1899 he states that he regards the vessels of the areas of Langerhans in the frog as venous.

Fig. 14 represents a very typical area from the pancreas of the *frog*, with its tall, narrow columnar cells with oblong, deeply stained nuclei arranged in

rather regular bands. Polyhedral cells with round or oval nuclei staining more faintly are scattered irregularly between the columnar cells and form more definite masses at the periphery. The sinusoids are distinct with simple endothelial lining and no connective tissue demonstrable, either surrounding the area or around the intrainsular sinusoids.

I have made no study of fish and reptiles, but Diamare and Harris and Gow and others have described large insulæ in reptilia, and Diamare and Rennie and others have found similar structures in fishes, the islands in these forms having the same structure as in the types studied by me; certain of the very large ones are, however, constant in position and, in some species of fish, are independent of the glandular tissue of the pancreas.

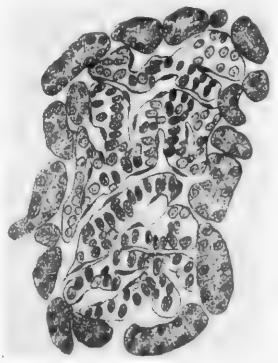


Fig. 14.—Section of area of Langerhans from pancreas of frog. × 200.

In general, then, it may be said that areas of Langerhans of very similar structure are found in all species of vertebrates that have been examined. In all species, they consist of cords or masses of epithelial cells derived from the same anlage as the pancreatic acini and sometimes retaining their connection with the glandular tubules. The cells vary somewhat in type in any one area, but the same or similar types are found in all the species examined, except the frog and bird; in the bird the cells are uniformly small and generally oblong, both nucleus and protoplasm staining poorly; in the frog, the predominant cells are tall and columnar with long deeply stained nuclei, the cells being packed together in single rows separated by blood-vessels. In all cases, the cords of cells are separated by large, irregular, anastomosing blood-vessels, having a complete endothelial wall and no or very little adventitia. In most forms, the endothelium seems to rest directly on the epithelial cells, and the connective tissue, when present, appears to be a secondary development, as in the somewhat sclerotic, adult human pancreas. The vessels correspond to Minot's definition of sinusoids. The

vascular network of the islands is made up of the windings, branchings, and anastomoses of several larger vessels of venous origin and large numbers of capillaries, which communicate intimately with the interacinar capillary plexus. The largest sinusoids are found in the central part of the islands, where the cords of cells are relatively small. The periphery of the areas is generally much less vascular. The largest areas found in my series were those of the rat, those of the guinea-pig being second. The reconstructions show that the cords of cells have the external form of branching and anastomosing tubules with occasional alveolus-like enlargements ; they are, however, solid structures with no lumen and no arrangement of the cells and nuclei which would suggest a lumen.

The areas are generally larger and more numerous in the central portion of the pancreas, especially in the bird, guinea-pig, and rat. In most species, the islands are intimately related to the glandular tissue, the capsule, when present, being so thin and delicate that it is demonstrated with difficulty. In the newborn child, however, most of the areas lie in the interlobular connective tissue and are surrounded by it; in the guinea-pig, the largest areas are in the connective tissue surrounding the larger ducts and are independent or practically independent of the pancreatic acini; and in some species of fish certain of the islands are very large, constant in location, and entirely independent of pancreatic tissue. In the human accessory pancreas, islands may or may not be present, the number reported in which they were absent being approximately equal to that in which they were present.

It is very natural to inquire concerning the function and meaning of an organ so constant in its presence and structure at all ages and in all species, occurring so early in embryonic life, and maintaining its vitality with so few changes through all conditions, and it is this side of the question which has interested the great majority of the investigators of the islands of Langerhans. The most varied views prevail. The discoverer, Langerhans, suggested the possibility of a relation to the nervous system, since these structures are often found in close proximity to the sym-

pathetic ganglia and the related nerves and staining reaction of their cells in ordinary dyes is not dissimilar. Because they are found in embryonic life and appear larger and more numerous in the embryo and new-born than in the adult, Gibbes, Harris and Gow, and others, regarded them as embryonal remains. Krause, Kühne and Lea, Renaut, Sokoloff, Dieckhoff, Pischinger, Pugnát, Mouret, and others basing their theory on the character of the cells, regarded them as lymphoid structures. Dogiel and Tschassownikow considered them as exhausted acini, or as Dogiel states "todte Punkte" because he found no connection with the duct and found fat globules in the cells.

Lewaschew, Laguesse, Mankowski, Statkewitsch, Pischinger, Saviotti, Kollossow, and many others assert that the islands are merely temporarily changed acini, which may change back into pancreatic acini. The reasons for this view are well expressed by Mankowski. He states that the number of islands increases during the period of gland activity and diminishes during rest; that the islands of Langerhans represent one of the morphological stages of activity of the pancreas. Every lobule of the pancreas must, at the end of its period of active secretion, pass into the "Stadium der Langerhans'schen Inseln," which represents the morphological phenomena of the greatest exhaustion or the greatest activity. He also notes the occurrence in any one section of various stages of transition between gland acini and the insulæ. Finally Zunz, Ssobolew, von Ebner, Schultze, Jarotzky, Sauerbeck, Diamare, and probably the great majority of the very recent writers on the subject regard the islands as independent organs, vascular glands, arising from the same anlage as the pancreas, but having a different function,—the elaboration of a secretion which is poured into the blood. This theory is based partly upon clinical and partly upon experimental data and will be considered more in detail later.

If perfectly fresh and well-fixed pancreas is examined, the cells of the islands, with their relatively small nuclei and prominent protoplasm and their arrangement into definite bands separated by sinusoids, are sharply differentiated from adenoid tissue, which can sometimes be seen in close proximity. The develop-

ment of the areas from an epithelial anlage also speaks against the theory of the lymphatic structure. That they are not embryonal remains may be readily seen from the fact that they do not in any sense degenerate in adult life, but remain alive, with a rich blood supply, and are, according to my experience, only relatively larger and more numerous in the young than in the adult. The rich blood supply, the absence of any appearance of degeneration, and the occurrence of dividing cells, as noted by Bizzozero and Vassale, by Schulze, and as I myself have seen, all refute the theory that they are pancreatic acini undergoing regressive changes. These dividing cells were especially numerous in the islands of the young child's pancreas, but some cells undergoing division were seen in nearly every section stained in Heidenhain's iron-lac-hæmatoxylin. The theory that the areas are being constantly derived from pancreatic acini and changing again into the glandular tissue is based (1), on the increase or diminution in the number and size of the areas during different conditions of digestion, and (2), on the occurrence of transitional forms, especially as shown by Lewaschew, after repeated pilocarpinization. Opie and Hansemann have repeated Lewaschew's experiments with pilocarpin, making careful counts of the islands, but have found no increase in the number of islands and no transitional forms. Ssobolew tested hunger, active digestion, and pilocarpin and found areas only slightly altered in number.

There is no question that structures do appear at times which might be interpreted as transitional forms. I have not infrequently seen groups of tubules in which the characteristic differentiation into two zones was entirely absent, the nuclei being centrally placed in the cells; these groups looked not unlike large islands of Langerhans, especially if, as sometimes happens, no lumen could be made out in the tubules. Sometimes, also, the eosinophile cells of the areas may be so numerous and so arranged as to resemble somewhat the tubules just mentioned. In the guinea-pig, I have once or twice seen, in apparent connection with one of the central areas, tubules having no outer basophile zone and having the nuclei centrally placed. These,

however, were very rare, and by tracing them through the series of sections, I was generally able to convince myself that they were merely contiguous with the areas and not continuous. The appearance of the pancreatic tubules varies so much under different conditions, and the relation of the tubules to the islands is, in most animals, so intimate, that it would not be difficult in almost any section to find structures which might be regarded as transitional. I have seen none, however, which could not be explained in some other and more rational way.

As to the size and number of the islands in different stages of digestion and under different diet conditions, Mankowski says that the areas increase during digestion, while Hansemann states that the apparent increase is due only to a more marked differentiation because of the change in the acini themselves.

To test the *changes produced in the islands of Langerhans by digestion and diet*, I have examined, measured, and counted a large number of islands from a considerable number of guinea-pigs under the following conditions: (1) normal in full digestion; (2) after different periods of time without food or drink; (3) after they had been kept on pure carbohydrate diet for different periods; (4) after the same periods on pure meat diet. As the guinea-pigs had to be forced to the meat diet, the earlier animals showed purely the changes of inanition, and I have not included them in my table. In order to make the conditions as nearly parallel as possible, I, in all cases except the first, killed the animal about fourteen hours after eating, divided the fresh pancreas into three approximately equal portions, fixed them in saturated bichloride solution; and cut the sections longitudinally in the direction of the main duct and through that portion of the gland which included the main duct. The sections therefore represented approximately the same regions of all the organs examined. I have measured sections of areas rather than the three dimensions, since the work previously reported has been based on the appearances noted in sections. The variations in size due to cutting through peripheral portions of the islands were overcome by measuring large numbers and taking the average. I counted and measured all the islands in the entire section in each case and in at least five sections, so that my average may certainly represent a true average for the areas in this region of the pancreas. While some qualitative changes were noted in the areas,—such as an increase or diminution of the number of eosinophile cells, a granular change in the cells, atrophy of cells with increase of intercellular substance,—there were none which could be regarded as constant for any one experiment and constantly increasing with the duration of the experiment. The table given below gives a brief synopsis of the main data gained from the counts and measurements. The largest number of very large islands was seen in the animal which had been kept on a meat diet for sixty days, while the proportion of island tissue to pancreatic glandular tissue is

constantly greater under meat diet than under carbohydrate diet or in hunger. But since, as may be seen from the measurements given of the normal areas, there are marked individual differences even under the same circumstances, I believe that this variation is due to individual peculiarities, although Ssobolew states that the size and number of the areas are quite constant for each species. In the following table, 1, in each case, represents the splenic third of the pancreas, 2, the middle third, and 3, the end nearest the duodenum.

Experiment.	Av. no. per mm.	Av. size in sq. mm.	Proportion of gland to island.	Size of largest area in sq. mm.	Size of smallest area in sq. mm.
Normal (full digestion)					
1.	.35	.02	1: .007	.31 x .38'	.04 x .06
2.	.42	.014	1: .006	.20 x .21	.04 x .06
3	.68	.021	1: .014	.21 x .43	.05 x .06
Hunger–36 hours					
1.	1.54	.012	1: .018	.13 x .37	.04 x .05
2.	1.22	.0064	1: .008	.19 x .20	.03 x .04
3.	.71	.014	1: .01	.24 x .36	.03 x .05
Hunger–46 hours					
1.	.346	.023	1: .008	.40 x .54	.02 x .04
2.	.4	.014	1: .015	.15 x .20	.03 x 11
3.	.18	.036	1: .006	.25 x .48	.05 x .06
Hunger–52 hours					
1.	.46	.018	1: .008	.19 x .52	.02 x .04
2.	.52	.015	1: .008	.19 x .23	.05 x .06
3.	.51	.009	1: .005	. 3 x .18	.03 x .04
Carbohydrate diet 30 days					
1.	.39	.018	1: .006	.18 x .33	.0 x .08
. .	.74	.013	1: .009	.38 x .40	.04 x .05
3.	.43	.019	1: .008	.20 x .35	.06 x . 07
Carbohydrate diet 60 days					
1.	.39	.016	1: .006	.19 x .21	.06 x .08
2.	.5	.019	1: .0099	.22 x .34	.05 x .06
3.	.52	.019	1: .0098	.18 x .50	.06 x .07
Meat diet, 30 days					
1.	.75	.015	1: .011	.13 x .50	.03 x .05
2.	.69	.015	1: .0102	.26 x .40	.05 x .06
3.	1.01	.012	1: .012	.17 x .19	.04 x .05
Meat diet, 60 days					
1.	.57	.032	1: .018	.30 x .68	.05 x .07
2.	.54	.025	1: .013	.27 x .42	.05 x .07
3.	.4	.031	1: .012	.22 x .68	.06 x .08

The so-called transition forms are merely resting pancreatic tubules. The size and number of the islands have individual variations under normal conditions fully as great as occur under varied conditions of digestion, hunger, and diet, and no changes occur under these conditions as has been shown by Opie, Schulze, Diamare, Jarotzky, and by myself. That the protoplasm of the insular cells, as Mankowski himself states, is chemically different

from that of the pancreatic cells is shown by the marked differences in their affinity for stains, especially for silver nitrate and safranin. As has been shown by many experimenters, the islands may be well preserved when the gland tissue is extremely atrophied, so that they do not seem to be subject to the same conditions as pancreatic cells. Of especial importance is the fact that islands have been found in some species independent or practically independent of the pancreas. For these reasons we certainly seem justified in the conclusion that the islands of Langerhans are independent organs with an independent function.

For over a century, cases of diabetes have been reported in which the pancreas was diseased, and the tendency has been constantly increasing to regard pancreatic disease as one, at least, of the causes of diabetes. This tendency has been greatly augmented since 1889, when Minkowski showed that in dogs the removal of the pancreas gave rise to glycosuria and most of the symptoms accompanying diabetes in man. Since then, medical literature has abounded with clinical and pathological reports as well as with chemical and experimental studies on the relation between the pancreas and sugar metabolism. After lesions of the islands of Langerhans in association with diabetes mellitus were described by Opie in 1900, discussion has been limited to the relation between sugar metabolism and the islands of Langerhans. I do not intend to enter in detail into the pathological reports, since my own work does not to any extent deal with the clinical side of the question and since pathologists are still at variance in their conclusions. Suffice it to say that Opie, Weichselbaum and Stangl, Wright and Joslin, Herzog, Ssobolew, and many others believe themselves justified in accepting the theory that pancreatic diabetes is due to some disturbance of the function of the areas of Langerhans, while Hansemann, Gutmann, Reitmann, Karakaschew, Herxheimer, Dieckhoff, Benda, and others contend that this theory does not explain and can not be based upon the facts observed.

Recently Sauerbeck has collected from the literature most of the published cases of diabetes in which the condition of the islands was reported and has added to them a number of his

own cases. He states that 117 of the 157 cases of the series show abnormal islands of Langerhans. In seven cases the islands could not be found. In one, they were represented by scar tissue. The changes described are as follows: (1) a diminution in size and number; (2) qualitative changes, such as hæmorrhage, fatty degeneration, acute and chronic inflammation, simple atrophy, hydropic degeneration (Weichselbaum and Stangl), sclerosis, and hyaline degeneration. If we reject as too indeterminate the purely quantitative changes, we still have 98 of the 157 cases in which qualitative changes were observed. Most of these changes have, however, in greater or less degree, been reported in non-diabetic cases, and eleven cases have been described by Dieckhoff, Hansemann, Litten, and Ziehl, in which the pancreas was entirely destroyed by suppuration or by carcinoma and yet no diabetes occurred, while non-diabetic cases have been observed in which no normal islands were found. Whether it be that in the complexity of function of the human organs, some other organ or tissue takes up the work of the areas under certain conditions, whereas in other cases they fail to do so, cannot be stated with any certainty. Several writers have asserted an interrelation of function between the pancreas, the liver, and the spleen, while Lorand has recently asserted that he has proved by experiments on dogs a relation between the function of the areas of Langerhans and the thyroid. He therefore states that the areas of Langerhans secrete a substance which neutralizes the poison produced by the thyroid and that diabetes may result either from the increased functional activity of the thyroid or from diminished activity of the areas of Langerhans. However this may be, all that can be said in the present condition of our knowledge is that the anatomo-pathologic investigation of this question has not as yet led to any satisfactory solution.

The announcement of the relation of the pancreas to sugar metabolism gave an impetus to an investigation of this question from the chemical point of view. Arnheim and Rosenbaum asserted the presence of a glycolytic substance in pancreas, in muscle, and in liver, the glycolysis being much increased if pancre-

atic extract were mixed with either the muscle or the liver extract. Šimáček claims to have isolated from the pancreas by precipitation with alcohol and ether a substance which, under anaërobic and aseptic conditions, causes an energetic alcoholic fermentation. Feinschmidt also obtains a glycolytic substance from liver, muscle, and pancreas, while Stoklasa has found a similar substance in plants and in numerous organs and fluids of the animal body, and Croftan claims to have found a glycolytic substance which he calls trypsin in the human blood. Stoklasa, Šimáček, and Feinschmidt find as the products of the glycolysis alcohol, carbon-dioxide, and acids. Cohnheim, however, claiming that this alcoholic fermentation is the result of bacterial influence, has published a series of articles in which he shows that there is present in muscle a glycolytic ferment, which, however, is inactive until acted upon by a substance in the pancreas which he calls an "activator." This substance is not destroyed by boiling and is soluble in water and alcohol but insoluble in ether. Increasing the amount of the pancreatic extract or of the ether precipitate of the same increases the influence up to a certain point, beyond which any further increase diminishes the glycolytic action. He regards this action as analogous to that of Ehrlich's amboceptor and complement. The presence of blood in the muscle causes glycolysis without addition of pancreatic extract and sometimes the glycolysis is diminished when pancreas is added. This fact would indicate the presence in the blood of variable amounts of the activator principle of the pancreas. He concludes that this substance is not a ferment since it is not destroyed by boiling, but is analogous to the other products of internal secretion, adrenalin, iodothyrin, and secretin. Fichera examined liver, muscle, cartilage, and epithelium of depancreatized dogs and found that the glycogen diminished and finally disappeared in about thirty days after the operation and concludes that the main phenomena of diabetes are a diminution of normal amylogenesis and a weakening and often disappearance of the glycolytic functions of the organs.

As has already been mentioned, in 1889 Minkowski succeeded in totally extirpating the pancreas of dogs, the operation being

followed by glycosuria and other symptoms of diabetes. Since then the operation has been repeated on dogs, cats, rabbits, birds, frogs, turtles, and eels, by numerous investigators, among whom may be mentioned de Dominicis, Minkowski, Harley, Kausch, Weintraud, Aldehoff, and Marcusi. While the results obtained by different investigators have varied somewhat, owing no doubt to the different methods used in the operation and also in the subsequent urinalysis, glycosuria following pancreas extirpation has been found in all the species examined, with the exception of ducks and geese, in which Kausch was able to demonstrate a hyperglycæmia but no glycosuria. De Dominicis found glycosuria in only about two thirds of the seventy animals (dogs, cats, rabbits, pigs, etc.) upon which he operated, but in all there were some changes such as polyuria, polyphagia, polydipsia, azoturia, phosphaturia, etc. Glycosuria was affected somewhat by diet, drugs, etc. Lüthje and others assert that the removal of the pancreas does not entirely destroy the function of glycolysis, since they found that the sugar disappeared from the urine while it was still present in the blood. Lépine and Thiroloix state that diabetes does not follow extirpation of the pancreas, if the animal is starved for some days before the operation. Pflüger, in order to test the constancy and permanency of glycosuria, removed the pancreas in a considerable number of animals, being careful that the extirpation should be total. He found that in every case glycosuria resulted and led to the death of the animal. He noted also that the liver always increased in size with an increase in fat, but that glycogen was absent.

After partial extirpation of the pancreas, the results are more variable and depend, as Minkowski thought, not so much on the size of the portion of the pancreas that is left as on its condition. The same seems to be true in most cases whether the remnant is left in situ or is transplanted under the skin, provided the blood supply is not interfered with.

In 1891, Vassale first noted the preservation of the islands and the glandular atrophy resulting from ligation and section of the pancreatic duct in rabbits. In 1898, Katz and Winkler found the islands of Langerhans preserved for some time after

the ligation of the duct. In 1900, Schulze tied off small portions of the pancreas in guinea-pigs, causing a complete atrophy of the ligated glandular portion, which was replaced by connective tissue, while the islands were unaffected. After eighty days he finds mainly connective tissue with a few dilated ducts and the normal areas of Langerhans. Since no glycosuria resulted from his experiments, he drew the conclusion that the areas of Langerhans are vascular glands of the type of the hypophysis, having an internal secretion whose function is probably to regulate the sugar content of the blood. In 1901, Mankowski repeated Schulze's experiment, tying two ligatures, and examining the portion between the ligatures, as well as the portion on either side of the ligatures. His results and conclusions were directly opposed to those of Schulze. He found both areas of Langerhans and pancreatic tubules atrophic and sclerosed as a result of his operation; as he notes an increase in the number of islands during digestion and a diminution in the resting stage of the pancreas, he decides that the islands are merely temporarily changed acini.

In 1902, Ssobolew ligated the duct and divided it between the two ligatures in dogs, cats, and rabbits. In cats, he found that the ducts generally reunited and became permeable before any extreme atrophy could take place. He found rabbits most favorable, since the duct could be tied and cut without injury to the pancreas. In the rabbit's pancreas, he found, as did Schulze in the guinea-pig, that the gland tissue became atrophic while the areas were well preserved up to the four hundredth day, at which time his observations ceased. At that time, the gland consisted only of the normal areas, surrounded by connective tissue containing the main duct. He describes the following changes in the glandular tissue: (1) There is loss of zymogen granules, protoplasm is homogeneous, and nuclei are irregular, shrivelled, and deeply stained. (2) Cells disappear and membrana propria collapses. (3) Centro-acinar cells are not seen after the eleventh day. (4) The changes in the glandular cells consist of atrophy, vacuolization, granular change, and sometimes indirect cell division. (5) Cells of small ducts increase and to some

extent also those of the medium-sized ducts. (6) Between the fourth and fifteenth days, there are mucous degeneration of cells of large ducts and thickening of walls. (7) After the thirtieth day there are atrophy of small ducts, collapse of membrane, and degeneration of epithelium. (8) New formation of connective tissue is interlobular, and there is much new elastic tissue around ducts and islands. (9) The number of blood-vessels diminishes after twenty days, the number of nerves after from sixty to seventy days; the number of ganglia and of cells in the remaining ganglia diminish and Vater-Pacinian bodies degenerate and do not reappear. (10) If measures were not taken to prevent it, there was a new formation of excretory ducts through which the secretion could be carried to the duodenum. Pawlow and Smirnow and Tiberti also note this regeneration of the duct and with it of the gland tissue, if the degeneration has not gone too far. (11) As to the areas, Ssobolew makes an important statement. While he finds the areas normal in the rabbit four hundred days after the operation, he finds that earlier, between the thirtieth and one hundred and twentieth day, some of the islands degenerate and many are reduced in size, but that there are individual differences in the power of resistance.

In none of the animals operated upon, did Ssobolew find glycosuria, but he does not state how frequently he examined the urine or how long after the operation he continued to do so. The point is of especial importance since Hédon in 1894 noted a slight transient glycosuria from the first to the fourth month after the pancreatic duct was filled with oil, thus causing atrophy of the gland. Sauerbeck (1904) calls attention to the same fact and also to its significance. He states that in rabbits (the same species on which both Hédon and Ssobolew operated) he noticed after ligation of the duct a slight glycosuria developing after the first month and at the same time a distinct alteration of the islands. This coincidence of facts is extremely suggestive for the island theory of diabetes.

Zunz (1905) after ligation of the excretory duct of the dog's pancreas noted the same atrophy of the gland parenchyma with preservation of the areas as described by Schulze, Ssobolew, and

Sauerbeck. Hansemann (1902), however, after ligating the duct in ten dogs, states that the atrophy extends only a short distance beyond the ligature and that the gland is later restored to a considerable extent. The islands may be well preserved or they may undergo fibrous degeneration. Lombroso (1905) tied and cut the duct in dogs and pigeons and found the glandular parenchyma practically unchanged. He states also that the islands show changes proportionate to those in the glandular tissue. It would seem to me probable that the inconsistent results obtained by these two investigators are due either to incomplete obstruction of the duct or to the regeneration of the duct which is so commonly met with, since their operations seem to have had so little effect on either the structure or the function of the pancreas. Lépine (1905) comes to the conclusion that the acini, as well as the areas of Langerhans, take part in furnishing the internal secretion to the blood and bases his conclusion on the following facts: (1) in some diabetics the insulæ are affected and in others, the glandular tissue; (2) after ligation of the pancreatic duct or injection of oil into it, the glycolytic power of the blood is greatly increased. As interference with the outflow of the secretion can affect only the gland parenchyma and not the islands, he considers this as sufficient proof that the acini as well as the islands form the glycolytic principle which is given to the blood.

Since the experiments of Schulze and Ssobolew and others showed that by ligation of the duct and interference with the outflow of the pancreatic secretion, a complete atrophy of the glandular acini of the pancreas was caused, while the areas of Langerhans remained unchanged, it seemed possible that by their method the areas of Langerhans might be isolated in considerable portions of the pancreas and the physiologic action of their extract determined; it was with this purpose that my experiments were undertaken. My experiments were performed in great part upon cats. Three were attempted upon guinea-pigs, but these were unsuccessful, as the pigs died during the first three days after the operation. In order to obtain as large an amount of extract as was consistent with the life and well-being of the operated

animal, I endeavored to pass my ligature as close as possible to the point of union of the two ducts. This point varies somewhat in different individuals, but in the main is about as represented in Fig. 15. In this figure, the ducts had been exposed

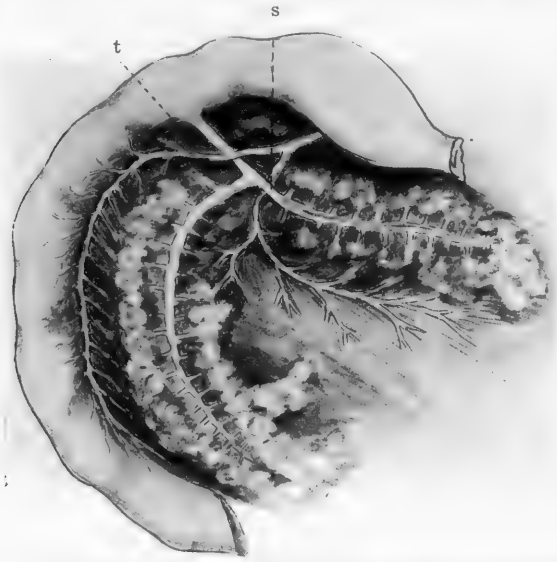


Fig. 15. Pancreas of cat laid open to show main ducts and their relation to the large vein near the junction of the ducts. The letters are explained in the text.

by dissection throughout their entire length, having been first filled with a blue solution. The blood-vessels had been previously injected with a red mass. The two halves of the pancreas were then laid back to expose the duct. The delineator has not represented quite accurately the relative size and length of the two portions of the gland, but the point of junction of the ducts and the relation of the ducts to the blood-vessels at the point of operation are nearly correct. My ligature, then, in five operations was at *t;* five glands were ligatured at *s* and *t*, and two at *s* only; ten had two ligatures tied tightly in the neighborhood of *s*, the pancreas being completely divided between the ligatures and the severed ends thoroughly cauterized to prevent the re-

union of the duct which had apparently taken place when the gland had been simply ligatured. In seven cases, after dividing the gland between two ligatures at *s*, a fold of omentum was drawn between the two ends and sewed or tied in place.

The animals lived after the operation from eighteen hours to 197 days. Ten of them, on account of an acute inflammation (three cases) or inanition (the cats often refused all food for the first week or ten days after the operation), died too early to hope for a sufficient degeneration of the gland tissue to attain even an approximate isolation of the islands. The changes found in the glandular parenchyma were much the same as those described by Ssobolew. The larger ducts were much dilated and filled with desquamated epithelial cells and detritus. The smaller ducts and the acini could scarcely be differentiated, since the gland cells had in the main early lost their differential staining power, the outer zone no longer taking the basic stain nor the inner the acid stain, but both staining alike. The cells in both small ducts and acini in many cases disappeared. The membranæ propriæ then collapsed and appeared like strands of connective tissue. The interlobular connective tissue was greatly increased, so that the lobules were much compressed, but still retained the appearance of lobules. In few, if any, of my cases has the lobule been entirely replaced by connective tissue except in the immediate neighborhood of the ligature, where often both gland tissue and areas of Langerhans had completely disappeared to give place to new connective tissue. This was especially the case in those animals in which the gland was divided and cauterized.

In my earlier operations, no special effort was made to avoid including the blood-vessel in the ligature and in some of these cases islands as well as gland tissue had suffered atrophy. In the other experiments, in which an effort was made to avoid interference with the blood supply of the gland, the areas were quite well preserved regardless of the extent of atrophy of the gland tissue. In those animals killed during the first six weeks or two months, however, many of the islands appeared smaller and less numerous than normal, although no accurate counts

and measurements were made. I at first considered this apparent atrophy as due to the poorer differentiation caused by the changes in the acinar cells, but they may represent the earlier and transitory atrophic changes noted by Sauerbeck, Ssobolew, and others, especially as in those animals that lived more than sixty days the areas appeared perfectly normal.

After some time, in some of my cases the duct became permeable again, as shown by passing a colored fluid through it into the duodenum, and in one case the enlarged and sclerosed duct broke through to the surface of the pancreas and so found vent for the secretion. In either case, there was a partial regeneration of the glandular tissue, and the regenerated tubules seemed to originate from the old, collapsed tubules that made up the atrophied lobule. The cells, though non-granular, flattened, non-functionating, and appearing like connective-tissue cells, had not lost the power of functionating and soon regained the appearance of secreting acini. In such glands, shown especially well in two experiments (No. XVI, 94 days and No. XXIII, 197 days), only two or three tubules with cells having granular protoplasm could be seen in a lobule section generally toward the center where the pressure of the connective tissue is less than in the outer part of the lobule, and where the rich blood supply of the areas of Langerhans may have helped to maintain the vitality of the cells. It was sometimes possible to trace a direct transition from the flattened and collapsed membrana propria which appeared merely as strands of connective tissue, through strands of cells containing a few granules to tubules staining like normal pancreatic acini. This easily and naturally explains the rapid regeneration of an extremely atrophied gland when an exit is made for its secretion. It may be to this method of regeneration that Ssobolew referred when he stated that in cats the ducts regenerated and the gland then soon regenerated, if the degeneration had not gone too far.

In most of the cases, I examined the urine during the first few days after the operation and again just before death. Sugar was not found in any of these cases, and as Sauerbeck's observations had not at that time appeared and I was unaware

of the possible occurrence of glycosuria after the first month, I did not continue the examinations long enough to be able to speak upon this point. In four of my cases, I tried the Mayo-Robson test on the urine just before death, with positive results in three cases and negative in one.

In all cases except when spontaneous death occurred during the night, the pancreas was removed immediately after the death of the animal and small pieces taken, one from the ligatured end, one from the neighborhood of the ligature, and one from the free end, and fixed in corrosive-sublimate solution for microscopic study. The rest of the gland, usually in two portions, was extracted either with glycerine or with water and its digestive and glycolytic powers tested. Starch digestion was tested with iodin and with Fehling's solution. Fibrin digestion was determined by the use of the bromine tryptophan reaction and the biuret test. Fat digestion was indicated by the reaction and by emulsification. The glycolytic power was tested by following as nearly as possible Cohnheim's method: a definite amount of glucose was added to a definite amount of muscle extract, and to this were added the pancreatic extract and also large amounts of toluol for its bactericidal action. In some cases, both boiled and unboiled pancreatic extracts, were used with the muscle extract and with the same results. The quantity of sugar was estimated, at once after mixing and again after twenty-four hours in the warm oven, with the ammoniacal copper solution of Pavy. Control tests with muscle alone and with pancreatic extract alone were carried on at the same time.

The results of these various *tests* are briefly indicated in the following table in which O indicates the mental end (usually the ligatured end), while D indicates the duodenal end (the free and hencemore normal end), of the pancreas. It will be noted from this table that seven of the twenty cases in which physiologic tests were made (and this included six of the ten cases in which the gland tissue was nearly normal) showed no digestion of starch, fibrin, or fat, while in several others the digestive action was very much weakened. The diminution and absence of digestive action are proportionate to the atrophic condition of the glandular tissue. In none of the cases, however, was there any appreciable weakening of the glycolytic or activator power of this extract, even when the glandular tissue was apparently atrophied

No.	Operation	Time	Glandular Parenchyma	Areas of Langerhans	Physiologic Tests				Other Organs.
					Starch	Fibrin	Fat	Sugar	
1	Ligature and section at *s.*	18 hrs.	Normal	Normal	Not tested				
2	Ligature, section, cauterization and omental insertion at *s.*	3 days	Hemorrhage near ligature	Normal	O. Pos D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
3	Three ligatures.	5 days	Vacuolation and compression of acini	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
4	Same as No. 2	6 days	Dilatation of ducts; compression of acini	Normal	No tests made				
5	Ligature and section.	6 days	No examination		No tests made				
6	Two ligatures at *s.*	7 days	Dilatation of duct; compression of acini	Small and poorly differentiated	No tests made				
7	Ligature, division, and cauterization.	8 days	Ducts dilated. Hemorrhage near ligature	Small; cells small and protoplasm degenerated	O. Pos D Pos.	Pos. Pos	Pos. Pos.	Pos. Pos.	
8	Ligatures at *s.* and *t.*	9 days	Vacuolation and compression of acini; necrosis and inflammation	Areas normal but small	No tests made				Liver; granular degen.
9	Ligature and section of duct at *s.*	10 days	Congestion and dilatation of ducts; acini compressed	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	Normal
10	Ligature, section, cauterization and insertion of omentum at *s.*	13 days	Hemorrhage near ligature	Normal	No tests made				Fat necrosis and hemorrhage
11	Ligature at *t.*	15 days	Atrophy and sclerosis of ligatured end	Some normal; some atrophied and sclerotic	No tests made				Kidneys: cloudy swelling, congestion and hyalin casts; liver: congestion, granular degeneration
12	Ligature, section, cauterization, and insertion of omentum at *s.*	16 days	Atrophy	Normal	No tests made				
13 14	Ligature, section, and cauterization at *s.*	27 days	Atrophy and sclerosis; dilatation of ducts	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	Normal
15	Ligature, section, cauterization, insertion of omentum at *s.*	49 days	Extreme atrophy and sclerosis	Normal; seem increased	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
16	Ligature at *t.*	35 days	Atrophy and sclerosis; dilatation of duct	Normal	O. Pos. D. Neg.	Pos. Neg.	Pos. Neg.	Pos. Pos.	
17	Ligature, section, cauterization, insertion of omentum at *s.*	59 days	Extreme atrophy and sclerosis; dilatation of duct	Normal except near ligature	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
18	Ligature and section at *s.*	87 days	Selerosis but little atrophy	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
19	Ligature at *t.*	90 days	Nearly normal	Normal	Pos.	Pos.	Pos.	Pos.	
20	Ligature, section and cauterization at *s.*	94 days	Extreme atrophy and selcrosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	

No.	Operation	Time	Glandular Parenchyma	Areas of Langerhans	Physiologic Tests. Starch	Fibrin	Fat	Sugar	Other Organs.
21	Ligature, section, and cauterization at *s*.	100 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	Liver: fatty degeneration
22	Ligature at *t*.	112 days	Slight sclerosis and atrophy	Normal	Pos.	Pos.	Pos.	Pos.	Liver: granular and fatty
23	Ligature at *s*.	114 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	
24	Ligatures at *s*. and *t*.	114 days	Regeneration; atrophy and sclerosis near ligature	Normal	No tests made				
25	Ligature at *s*	119 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
26	Ligatures at *s* and *t*.	126 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
27	Ligature and section at *s*.	156 days	Atrophy and sclerosis	Normal	O. Pos. D. Pos.	Pos. Pos.	Pos. Pos.	Pos. Pos.	
28	Ligature section, and cauterization at *s*.	197 days	Extreme atrophy and sclerosis	Normal	O. Neg. D. Pos.	Neg. Pos.	Neg. Pos.	Pos. Pos.	

The following table of the glycolytic tests in the seven cases in which there was no digestive action will indicate the very slight change, if any, in the activator power of the pancreatic juice caused by the extreme atrophy of the pancreatic acini. The slight difference is probably due to the smaller amount of the "activator principle" used in the case of the atrophied portion of the gland. (O, indicates omental and D, duodenal end of pancreas.)

No.	Time.	Original amount of sugar in grm.	Amount of sugar after 24 hours in grm.	Actual loss in grm.	Per cent. of loss.
15.	49 days.	O 2.0776	1.315	0.762	.7%
		D 2.0776	1.209	0.868	.8%
16.	53 days.	O 4.25	3.67	0.58	.6%
		D 4.25	3.57	0.68	.7%
17.	59 days.	O 1.66	0.83	0.83	.83%
		D 1.66	0.81	0.83	.81%
20.	94 days.	O 0.76	0.4	0.36	.12%
		D 1.13	0.77	0.25	.1%
21.	100 days.	O 1.0125	0.27	0.74	.8%
		D 0.875	0.215	0.66	.9%
23.	114 days.	O 0.69	0.21	0.48	.5%
		D 0.93	0.29	0.64	.6%
28.	197 days.	O 1.5	1.15	0.25	.41%
		D 1.13	0.77	0.36	.42%

The experiments have not proven so decisive as I had hoped, and for two reasons: (1) the isolation of the areas was not always

complete; and(2) the insulæ in the cat's pancreas are generally small and it is difficult to get, even from the larger omental portion of the pancreas, when atrophied, sufficient extract to make very many satisfactory tests. I feel sure, however, that the chemical and physiological examination of isolated island tissue offers the only certain means of deciding the difficult questions regarding the function of these structures. While my results must be regarded as relative rather than absolute, they speak with no uncertain voice in favor of the theory that the islands manufacture a substance, analogous to the "activator principle" described by Cohnheim, which favors the glycolytic action of muscle ferment. To make these results more certain, it is necessary to use the isolated island tissue in large enough amounts so that numerous tests may be made not only of its activator action on muscle ferment, but also its effect on blood pressure and, most important of all, its influence on the experimental diabetes of depancreatized animals and on human diabetics. It is a question whether the operative method can in any animal furnish the isolated island tissue in large enough amounts for these purposes.

Rennie, however, has recently described two species of fish (*Lophius piscatorius* and *Scorpæna scropha*) in which one large "principal islet" and several smaller islands of structure analogous to that of the areas of Langerhans in mammalia may be distinguished with the unaided eye and may be dissected out entirely free and isolated from pancreatic tissue. If these shall prove to have the same function as the areas of Langerhans in mammalia and other forms, they offer the opportunity for further and more satisfactory investigation along these lines. Since my experimental work was finished and had been reported in part at the session of the Association of American Anatomists at Philadelphia in 1904, two preliminary reports have appeared, one by Rennie himself and one by Diamare and Kuliabko, announcing that they are now following out a similar line of investigation on the isolated islands of these two species of fish. The results given in their preliminary reports are by no means decisive or very satisfactory. Rennie reports three tests, two

qualitative and one quantitative, on a glucose solution with an extract of these areas. The result would seem to show that this extract does not *alone* have the power of inverting grape sugar. He hints, however, that his experiments with this extract on diabetic patients have been more favorable to the island theory of diabetes. Diamare and Kuliabko, on the other hand, tested in several cases the action of this island extract on starch solution and on glucose solution. They were able to show, so far as their few tests made can decide, that these islands have no power whatever to digest starch. While the starch solution with the pancreatic extract from the same fish gave with iodine no starch reaction, and with Trommer's test gave marked sugar reaction, that with the island extract gave only the starch reaction with iodine. Their test for the glycolytic action is much less decisive, since no change was noted until after forty-eight hours, so that the inversion may have been due to bacterial action; further experiments, therefore, are necessary. The outline which they give of the work which they are now carrying on shows that they are carefully investigating the chemical and physiological characteristics of these isolated areas of Langerhans in the fish, and we shall hope for much light on this question when their work shall have been completed and their results published.

In conclusion it may be stated that there occur in the pancreas of all vertebrates homologous structures known as areas of Langerhans.

These have the structure of vascular glands with a sinusoidal circulation and are not changed during the secretory activity of the pancreas. They also remain unaltered when the glandular acini become atrophied as a result of ligation, section, or obstruction of the duct.

They have a secretion which is probably poured into the blood-vessels; this secretion has no digestive action on starch, fibrin, or fat, but has a marked glycolytic function, especially when added to muscle extract and is therefore analogous to the "activator principle" described by Cohnheim.

In closing I am glad to acknowledge my indebtedness to

Professor G. Carl Huber for many helpful suggestions received during the progress of this investigation.

EXPLANATION OF PLATES.

PLATE XII.

Fig. 1.—Wax reconstruction of typical island of Langerhans from pancreas of rat. × about 200.

Figs. 2 and 3.—Wax reconstructions of islands of Langerhans from pancreas of rabbit. P, connection with pancreatic tubules. × about 200.

PLATE XIII.

Figs. 1 and 2.—Wax reconstructions of areas of Langerhans from pancreas of cat. × about 200.

Figs. 3, 4, 5, and 6.—Wax reconstructions of injected blood-vessels of areas of Langerhans from pancreas of cat. × about 200.

PLATE XIV.

Fig. 1.—Wax reconstruction of area of Langerhans from human pancreas. × about 250.

Fig. 2.—Wax reconstruction of blood-vessels with surrounding connective tissue in same area, the cellular cords of which are reproduced in Fig. 1. × about 250.

PLATE XV.

Fig. 1.—Interior view of model reproduced in Fig. 1, Plate XIV, Fig. 1. × about 200.

Fig. 2.—Interior view of model reproduced in Fig. 2, Plate XIV, Fig. 2. × about 200.

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Fig. 1.

Fig. 2.

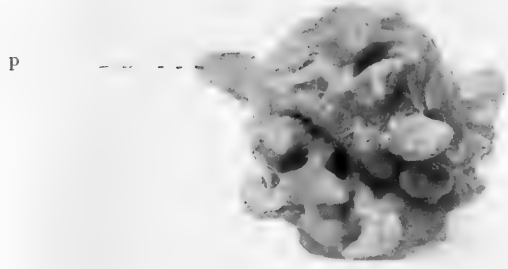

Fig. 3.

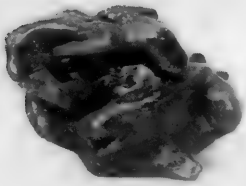

Fig. 1.

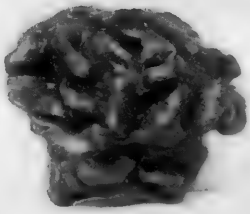

Fig. 2.

Fig. 3.

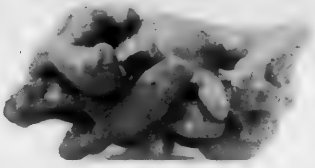

Fig. 4.

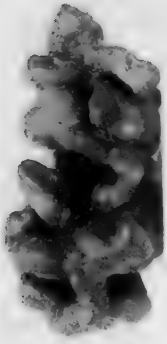

Fig. 5.

Fig. 6.

Fig. 1.

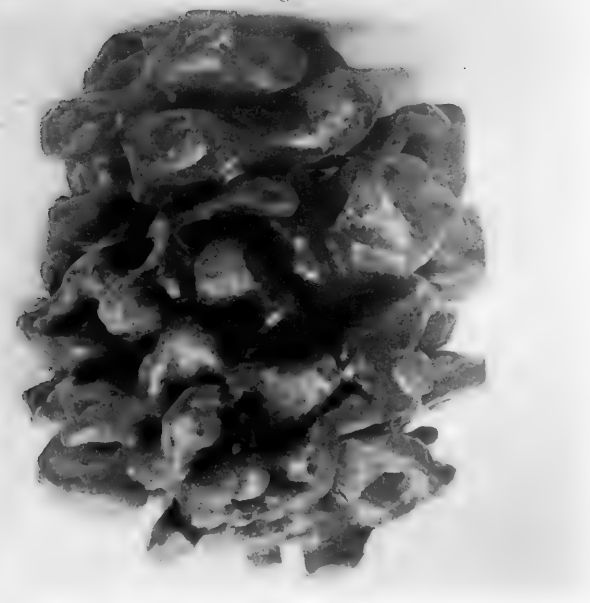

Fig. 2.

Fig. 1.

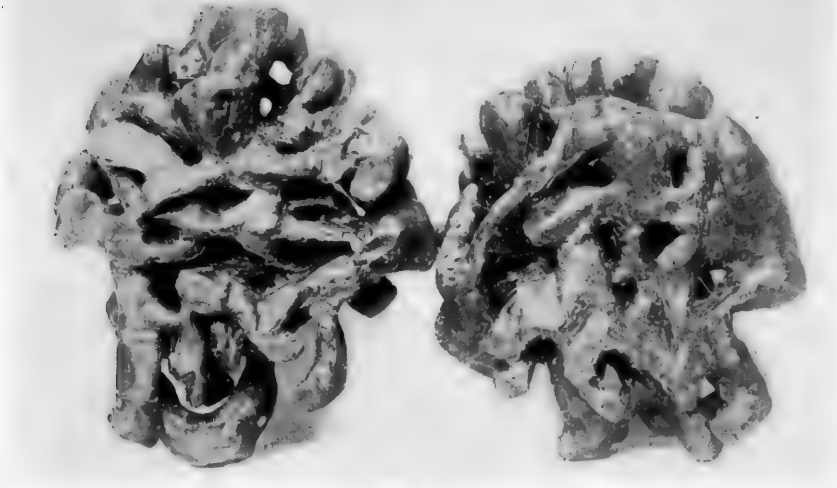

Fig. 2.

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THE EFFECT OF INTRAVENOUS INJECTION OF SOLUTIONS OF DEXTROSE UPON THE VISCOSITY OF THE BLOOD.[1]

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The viscosity of the blood is expressed in this paper in terms of the coefficient K, obtained by calculation from the following known factors: the quantity of blood collected during a definite period, its specific gravity, the blood pressure, and the length and internal diameter of the capillary tube through which the blood is allowed to flow. When this coefficient has been compared with K for distilled water at 37° C., the value of which has been proved by Poiseuille to be 4700, it is possible to express the viscosity of the blood in terms of multiples of the viscosity of distilled water, heated to the temperature of 37° C. The method by means of which the aforesaid factors were obtained has been fully described by Hürthle.[2]

In the experiments now to be recorded, I have made use of four capillary tubes, numbered II, III, IV, and V. Tube No. II was used in Experiments 3, 4, 5, and 6; No. III in Experiment 1; No. IV in Experiment 2, and No. V in Experiment 7. The accuracy of each capillary was tested beforehand by a series of experiments with distilled water. Their dimensions were as follows:

[1] This study has been conducted under a grant from the Rockefeller Institute for Medical Research.

[2] Hürthle, *K. Arch. für die ges. Phys.*, 1900, lxxxii, 415.

Capillary	Length	Diameter
	mm.	mm.
II	213.0	0.6636
III	245.5	0.6636
IV	299.2	0.4100
V	331.7	0.7150

The experiments were performed upon dogs under light ether narcosis. The method remained the same in all cases. The normal viscosity having been tested by a number of determinations, a certain quantity of a concentrated solution of dextrose, heated to body temperature, was slowly injected into the facial vein. After an interval lasting from ten to fifteen minutes three or four additional determinations were made, fifteen minutes apart.

The details of the experiments with the concentrated solution of dextrose are given in Table I. In Experiments 1 and 2 only 5 c. c. of the solution were injected, while in the last two, 50 and 100 c. c. respectively were administered. The determinations in each experiment fall into two groups, which show respectively the normal viscosity and the viscosity prevailing after the injection.

On comparing the coefficients of the viscosity with one another, it is found first of all that their value does not remain constant. In the first two experiments, K became smaller after the injection, but in the third and fourth experiments larger than normal. As the actual change in the viscosity is inverse to the numerical change in the coefficient, these facts imply that the viscosity of the blood is increased by small and decreased by large quantities of the solution. The increase which occurred when the quantity of solution injected was small is only a moderate one, and is entirely in accord with the fact[3] that small amounts of pure distilled water, even when used alone, render the blood slightly more viscous. Larger quantities of the solution of dextrose, on the other hand, produced an im-

[3] Burton-Opitz, R., *Jour. of Phys.*, 1904, xxxii, 8.

TABLE 1.

THE VISCOSITY OF THE BLOOD AFTER INTRAVENOUS INJECTION OF DEXTROSE.

Number of exp. and weight of dog		Deter. No.	Quantity of sol. inj. c. c.	Time elapsed since inj. min.	Temperature of animal °C.	Specific gravity of blood	Quantity mg.	Time sec.	Pressure mm. Hg.	Viscosity coefficient K	Difference between K of same group	Mean value of K	Change in K equals
Exp. 1 13.0 K.	Normal	1	—	—	36.8	1.0547	2615.1	18.24	179.8	957.24	59.46	986.97	103.70
		2	—	—	"		1865.2	14.31	153.9	1016.70			
	After inj.	3	5	15	"	1.0564	2092.7	16.71	174.2	861.60	51.70	883.27	
		4	—	30	"		2510.4	20.53	167.5	874.93			
		5	—	45	"		2374.4	18.30	170.2	913.30			
Exp. 2. 17.5 K.	Normal	1	—	—	36.5	1.0589	233.7	22.22	138.1	761.56	29.86	746.63	37.70
		2	—	—	"		250 9	24.74	138.6	731.70			
	After inj.	3	5	15	"	1.0594	168.8	22.53	106.8	701.14	15.59	708.93	
		4	—	30	"		200.3	22.31	125.2	716.73			
Exp. 3 12.0 K.	Normal	1	—	—	37.0	1.0522	2662.9	17.46	154.8	1028.42	77.03	1035.69	51.24
		2	—	—	"		2130.8	14.90	138.5	1077.85			
		3	—	—	"		2568.0	18.60	144.0	1000.82			
	After inj.	4	50	15	"	1.0504	2896.5	16.25	159.6	1167.80	146.35	1086.93	
		5	—	30	"		3007.4	19.32	151.9	1071.54			
		6	—	45	"		2686.3	20.25	135.8	1021.45			
Exp. 4 13.0 K.	Normal	1	—	—	36.8	1.0544	1915.0	20.07	115.4	861.26	41.83	851.14	95.92
		2	—	—	"		2016.3	19.84	122.1	867.00			
		3	—	—	"		1685.9	18.41	115.6	825.17			
	After inj.	4	100	10	"	1.0519	2471.7	21.54	121.1	989.38	79.28	947.06	
		5	—	20	"		2724.7	25.43	118.8	941.70			
		6	—	30	"		2362.7	23.53	115.2	910.10			

mediate decrease corresponding to the amount of solution injected. The specific gravity of the blood pursued, in these experiments, a course parallel to the vicosity.

It is also to be noted that the viscosity did not retain its new value for any length of time, but assumed with each successive determination a value closer to the normal. The greatest change was observed, therefore, shortly after the injection, while thirty to forty-five minutes later, when osmotic interchanges between the blood and the tissues had taken place, the difference had become much less evident.

Three additional experiments were performed with a view to determine the viscosity during hyperglycæmia, this condition having been brought about by painting the surface of the pancreas with a solution of adrenalin. A number of determinations of the viscosity were made before as well as after the adrenalin had been applied. In all three cases traces of sugar were found in the urine after the completion of the last determination. The details of these experiments can be readily obtained from Table II.

It is clearly brought out in the foregoing table that the viscosity of the blood during hyperglycæmia is somewhat greater than normal; however, the increase did not amount to more than seventy points in the experiments now recorded. Thus, if Experiment 7 be taken as an example, we find that the viscosity, which was in this case 6.6 times as great as that of distilled water at 37° C., became 7.0 times as great after the use of the adrenalin. Corresponding changes were noted in the specific gravity of the blood.

TABLE II.

THE VISCOSITY OF THE BLOOD AFTER APPLICATION OF ADRENALIN TO PANCREAS.

Number of exp. and weight of dog		No. of determ.	Temp. of animal	Specific gravity of blood	Quantity mg.	Time sec.	Pressure mm. Hg.	Viscosity coefficient K	Difference between K of same group	Mean value of K	Change in K equals:
Exp. 5	Normal	1	36.5	1.0488	1333.4	13.94	96.1	1042.35	39.85	1062.95	53.48
		2	"		1853.2	15.54	115.4	1082.20			
		3	"		1672.3	14.51	113.4	1064.32			
9.5 K.	After applic. of adrenalin	4	"	1.0494	1778.7	16.15	113.5	1015.60	16.50	1009.47	
		5	"		2338.8	17.94	134.6	1013.72			
		6	"		128.59	15.83	85.1	999.10			
Exp.6	Normal	1	36.5	1.0610	1900.9	14.37	171.1	800.32	22.05	801.83	47.37
		2	"		2269.6	14.96	189.5	813.62			
		3	"		1632.0	15.18	140.6	791.57			
10.0 K.	After applic. of adrenalin	4	"	1.0614	1462.7	12.08	165.4	757.55	6.17	754.46	
		5	"								
		6	"		1482.0	12.20	167.3	751.38			
Exp. 7	Normal	1	36.9	1.0559	1100.5	16.54	111.1	719.85	14.87	710.02	66.32
		2	"		1344.5	18.31	125.2	704.98			
		3	"		1631.6	13.40	207.5	705.23			
9.5 K.	After applic. of adrenalin	4	"	1.0573	1511.2	15.84	183.8	623.11	34.43	643.70	
		5	"		1582.1	15.09	182.6	650.46			
		6	"		1399.1	16.64	153.5	657.54			

EFFECTS OF INTRAVENOUS INJECTIONS OF RADIUM BROMIDE.

By R. BURTON-OPITZ and GUSTAVE M. MEYER.

(From the Laboratories of Physiology and Physiological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.)

Plate XVI.

The present study was undertaken with a view of determining in a general way the effects of intravenous administration of radium upon the circulation and respiration. The problem was suggested to us by Dr. William J. Gies, under whose guidance a number of researches, dealing with the more extensive question of the action of radium upon animal and vegetable cells, have recently been carried on in the laboratories of Columbia University.[1]

For the radium used in these experiments we are greatly indebted to Mr. Hugo Lieber. It was supplied to us in the form of the bromide, in preparations of 240, 1000, and 10,000 activities. The strength of the solution used was the same in all cases. It contained 45 mg. of the dry substance in 25 c. c. of the solvent; each cubic centimeter of the solution, therefore, contained 1.8 mg. of the radium preparation.

The amount of the radium present varies directly with the radio-activity. Preparations of 1,500,000 activity are said to represent pure radium bromide.[2] Taking this figure as the standard of purity, 1.8 mg. of the radium preparation of 10,000 activity contained approximately only 0.0126 mg., the same quantity of the preparation of 1000 activity contained 0.00126 mg. and a like amount of the preparation of 240 activity only 0.00036 mg.

[1] Preliminary communications of the results were made last May. See Gies and collaborators: *Proceeding of the Society for Experimental Biology and Medicine*, 1905, ii, 86; also, *Science*, 1905, xxi, 986; *American Medicine*, 1905, ix. 1026; *Medical News*, 1905, lxxxvii, 520.

[2] Rutherford, *Radio-activity* (Second Edition), 1905, p. 14, "No definite results have yet been given on the activity of pure radium, but the Curies estimate that it is about one million times that of uranium and may possiby be higher."

of actual radium bromide. Expressed in terms of the element radium (atomic weight = 225), each cubic centimeter of the solutions of the different preparations used in these experiments represents respectively about 0.00739, 0.00074, and 0.000178 mg. of radium. It must also be mentioned beforehand that the effect of these radium preparations may be due not alone to the radium, but to its disintegration products, namely, emanation,— radium A, radium B, radium C — or to the radiations given off by these. As will be brought out later, there appears to be a distinct difference between the action of the perfectly fresh solution and one that has been left standing for some time in an open receptacle. Whether this difference is due to an increased or decreased amount of emanation in the solution has not been determined as yet.

The substance was dissolved in distilled water, perfectly fresh solutions, heated to body temperature, being employed for the injections. The experiments were performed upon dogs weighing from three to seven kilos. Light ether narcosis was employed. The blood pressure was recorded by a mercury manometer connected with the carotid artery and the respiratory movements by means of a modified pneumographic arrangement. The injections were made into the facial vein.

A number of preliminary tests were made to determine the influence of the intravenous injections of such quantities of distilled water as were made use of subsequently in the experiments with radium bromide. Only in those cases in which the injection was made with more than a moderate degree of rapidity could any effect upon the circulation be detected.

The radium bromide of 240 activity was employed in six experiments, approximately 10 c. c. of the solution, or 18 mg. of the preparation being administered in each case. Curve I, (Plate XVI) which forms only a small part of the entire record of Experiment I, may serve to show the character of the changes observed. It was obtained from a dog weighing four kilos and differs in no particular from the other records of this series. The injection began at A and was discontinued at B. Besides

the blood pressure (Pr) the curve also includes a record of the respiratory movements (R) and a time-tracing (Sec).

The blood pressure began to rise in all cases shortly after the beginning of the injection and continued to increase gradually until about 5 c. c. of the solution had been injected, or during a period of from thirty to thirty-five seconds. The length of the period intervening between the beginning of the injection and the appearance of the rise depended somewhat upon the rapidity with which the solution was injected. On the whole, however, it was rather brief, averaging about four or five seconds. The rise itself appeared as a rule rather abruptly and attained its maximum value in the course of about thirty-five seconds. The maximum increase here recorded amounted to 60 mm. Hg and the minimum rise to 20 mm. Hg, the average noted in the six experiments being 35 mm. Hg.

By comparing the normal frequency of the heart with its rate during the period of increased blood pressure, only very insignificant differences were brought out. If anything, a hardly recognizable inhibition had taken place, which, however, was more than compensated for by an increase in the force of the cardiac contractions. A study of the curves proves beyond question that radium bromide of this activity exerts a moderate tonic action upon the heart muscle. However, the greater force of the contractions is not at all sufficient to explain the marked increase in blood pressure. For this change, no doubt, a direct vaso-constrictor influence of the radium bromide upon the musculature of the blood-vessels must be held responsible.

The gradual rise in blood pressure, caused, as has just been stated, by a general constriction of the blood-vessels, was invariably interrupted after about thirty-five seconds by a very pronounced irregularity and inhibition of the heart. Relatively long diastolic periods were now followed in rapid succession by periods during which a number of short forcible contractions caused the mercury to rise far beyond any ordinary level, but in spite of the fact that the blood pressure attained at times quite extraordinary heights, its average value remained as a rule well below the height to which it had risen during

the period of vaso-constriction. As the experiment progressed, the pressure gradually became lower and lower and sank to zero shortly after the onset of the respiratory paralysis. It happened at times that the heart regained for a period of indefinite length a certain regularity of contraction, but even during these moments of partial restoration its frequency was very much less than normal.

A change in the rate of respiration during the period of vaso-constriction could not be detected. Shortly after the onset of the cardiac symptoms, however, the respiratory movements became distinctly less frequent and deep. As the experiment progressed, this inhibitory influence came more and more into prominence, until finally a complete respiratory paralysis resulted. The heart ceased beating shortly after the respiratory movements came to a standstill. When the lungs were inflated artificially soon after the onset of the respiratory paralysis, the heart action continued; respiration, on the other hand, did not become automatic again during the time of these experiments, i. e., within about ten minutes after the cessation of the natural respiratory movements.

Two experiments were made in addition upon dogs whose vagi had been divided beforehand. A difference in the effect of the radium bromide, directly ascribable to this operative measure, could not be detected, excepting, of course, a lesser prominence of the initial vaso-constrictor reaction. The latter change was due entirely to the pre-existing high arterial tension and blood pressure. Vagus stimulation remained effective throughout the experiment. These facts, therefore, tend to prove that the radium bromide does not exert a central, but rather a direct influence upon the substance of the heart.

Several tests were also made with radium bromide solutions that had been left standing in an open flask for about one week preceding the day of the experiment. It was found in all cases that these solutions had lost a good deal of their effectiveness.

The details of the experiments with radium bromide of 240 activity are given in the following table in conjunction with those

in which the preparations of 1000 and 10,000 activities were employed. Besides the weight of the animal and the amount of radium administered in each case, the table also gives comparative figures regarding the cardiac and respiratory frequency and the blood pressure during the different periods of the experiment.

THE EFFECT OF INTRAVENOUS INJECTION OF RADIUM BROMIDE SOLUTIONS.

	Exp.	Weight of dog K.	$RaBr_2$ injected. per mg.K	Normal			Period of constriction			Period of irregular heart action		
				Resp. rate	Pulse	Blood pr.	Resp. rate	Pulse	Blood pr.	Reps. rate	Pulse	Blood pr.
	1	3.4	5.3	9	90	88.6	8½	90	108.9	cessation	very irreg;	cess.
	2	5.0	3.6	12	85	77.5	12	85	121.0	"	"	"
$RaBr_2$	3	3.7	4.9	9	74	122.5	10	75	182.8	"	"	"
240 act.	4	4.3	4.2	11	78	103.7	11	76	146.0	"	"	"
	5	5.7	3.2	8	95	126.2	8	89	152.1	"	"	"
	6	4.6	3.9	12	82	114.9	12	82	139.3	very slow	"	"
$BaBr_2$	7	4.0	2.5	14	80	136.8	13	78	169.0	cessation	"	"
1000 act.	8	5.5	3.9	12	76	132.7	12	76	152.2	"	"	"
	9	5.5	3.3	16	82	140.1	17	82	175.7	9	36	87.6
$RaBr_2$	10	5.2	3.6	17	86	114.8	17	82	126.5	10	47	102.2
10,000act.	11	7.0	3.6	13	91	97.7	14	91	132.1	8	31	88.7
	12	5.5	3.4	18	80	122.1	17	80	135.6	8	41	75.2

As barium bromide forms so large a part of the radium bromide preparations, it was thought possible that the effects noticed in the preceding experiments might be due at least in part to this constituent. To test this assumption several experiments with pure barium bromide solutions were performed, the method being the same as the one described in an earlier paragraph of this paper.

Our results relative to the barium are entirely in harmony with those described by Mickwitz,[3] Böhm,[4] and Bary.[5] Differences in the degree of the reaction were observed by the first two authors in accordance with the strength of the barium solution employed. When small doses were given (0.01 gm.) only slight rises in the blood pressure were observed. After larger doses (0.02 gm.) the increase in the blood pressure was followed by an irregularity of the heart, while still larger amounts (0.04 gm.) caused in addition a rapid fall in blood pressure and the death of the animal. Bary corroborated these results and

[3] Mickwitz, Dissertation, Dorpat, 1874.

[4] Böhm, *Archiv f. exper. Path., u. Phar.*, 1875, iii, 216.

[5] Bary, Dissertation, Dorpat, 1888.

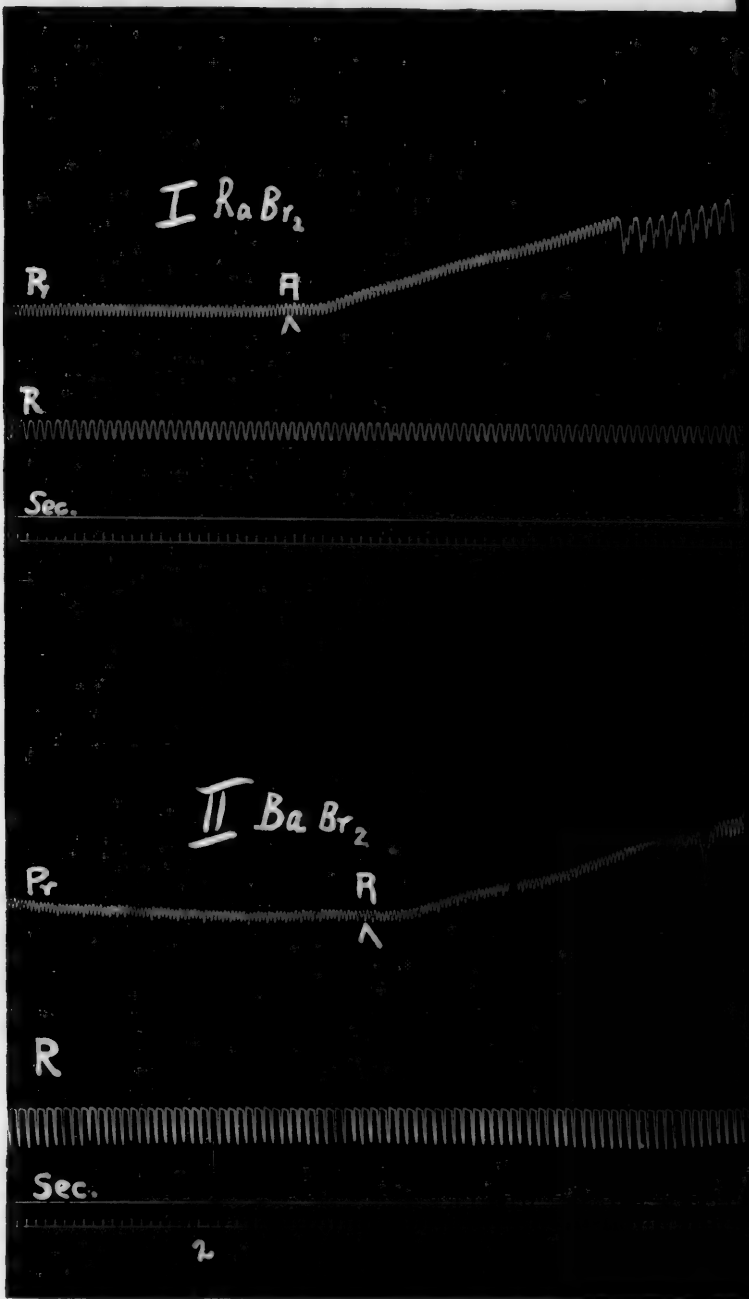
showed that the rise in blood pressure is due to a direct action of the barium upon the musculature of the blood-vessels and a slight tonic action upon the cardiac muscle.

However, in order to bring the great similarity in the action of barium bromide and radium bromide of this activity more convincingly to the attention of the reader without entering upon a lengthy description, it has been thought best to reproduce underneath Curve I a part of a tracing obtained during one of the experiments with barium. This record does not differ in any important particular from those of the other barium experiments and, moreover, on comparing it with Curve I, it would be difficult to detect fundamental points of difference in the action of these two elements.

It is entirely probable, therefore, that the effects previously attributed to the radium preparation are due in a large measure to its main constituent, barium. In accordance with this assumption we might, moreover, suppose that these circulatory and respiratory changes would be less evident after the administration of radium of higher activity, unless radium showed more markedly the effects exhibited by barium, a possibility suggested by the chemical similarity of these two elements.

To test this inference, we performed, in addition, two experiments with radium bromide of 1000 activity and four experiments with radium bromide of 10,000 activity; proportionately equal amounts of the preparation were injected.

The results derived from the experiments with the radium of 1000 activity differed practically in no way from those obtained with the radium of 240 activity. Very definite changes were observed, however, when the preparation of 10,000 activity was employed. The initial vaso-constrictor reaction lost a large share of its former conspicuousness and abruptness, while the series of short forcible contractions of the heart which often caused the blood pressure to rise suddenly in jerks beyond any ordinary level were now replaced by slow pulsations of nearly equal amplitude. The average blood pressure remained well below the normal. The most prominent symptoms, therefore, were the vaso-constriction followed shortly afterward by a marked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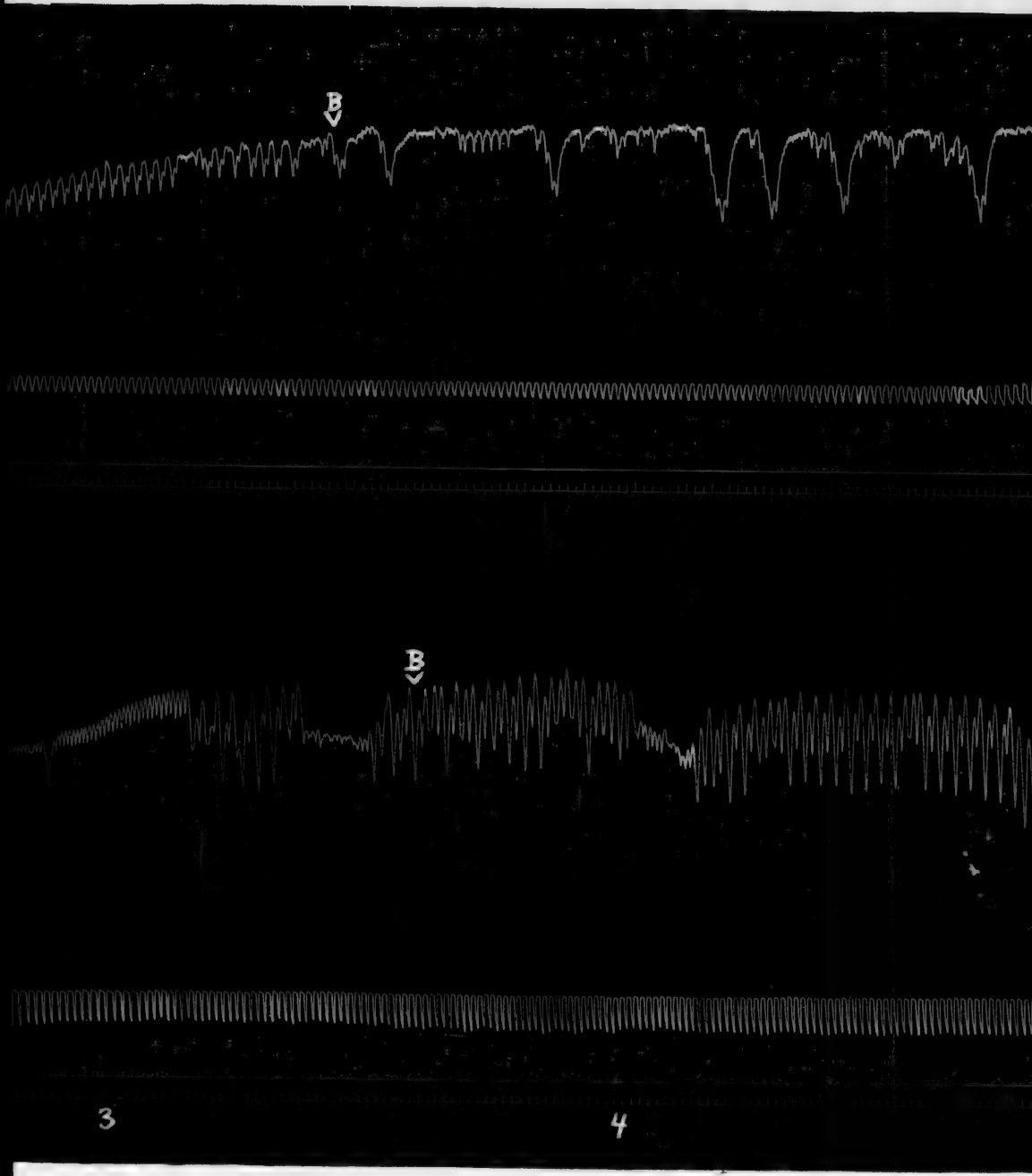

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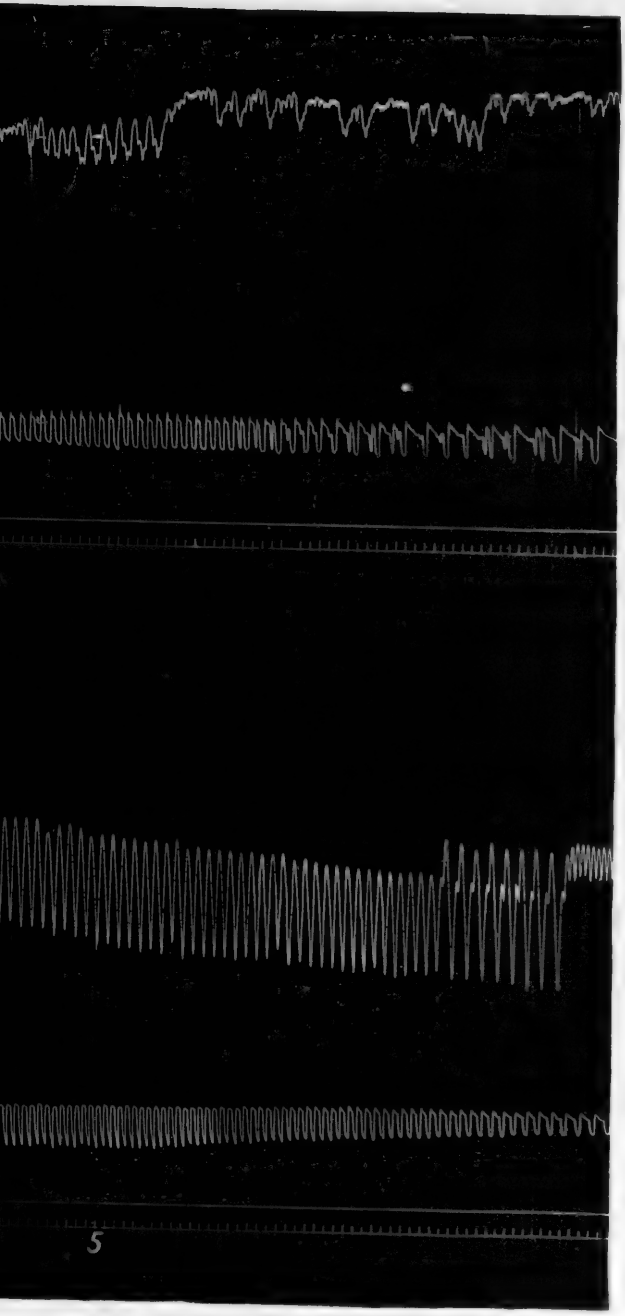
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cardiac inhibition, but without any marked irregularity of contraction. The inhibition began during the injection of the solution and continued for an indefinite period, a complete restoration of heart action being the usual result. These effects could be produced a second and third time by renewed injections of the solution; it was found, however, that constantly larger quantities had to be used, a fact favoring the view that a certain adaptation of the body to the radium bromide does take place.

The injections were also followed by a moderate decrease in the frequency and depth of respiration, the respiratory inhibition being most pronounced during the period of cardiac inhibition. However, a complete respiratory paralysis, as in the previous experiments, did not result.

While the injections of radium bromide of 240 and 1000 activities proved fatal with only one exception, the radium bromide of 10,000 activity did not produce fatal results within the time of the experiments. It seems to be established, therefore, that the injurious effects of the radium bromide preparations used were due in a large measure to the accompanying barium salt,—at least, the evidence here brought out points to that conclusion. Naturally, complete proof of this statement can be obtained only by the use of chemically pure radium salts, i. e., such as show activities of 1,000,000 or more.

THE PHOTODYNAMIC ACTION OF EOSIN AND ERYTHROSIN UPON SNAKE VENOM.

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The study of the destructive action of photodynamic substances upon cells, toxins, ferments, and other chemical bodies, has opened up an interesting field of biological and chemical research. This paper will deal with the effects of two active photodynamic chemicals—eosin and erythrosin—upon the toxic principles of venom.

The starting-point of this study is the complex nature of snake venom, with its several independent toxic principles The effects of these principles are clearly demonstrable by biological tests *in vitro* and in the animal body. Since the action of snake venom has been extensively and profitably studied, the properties of the principles have been fairly established.[1] As regards their chief activities these principles can be divided into the following: neurotoxines, hæmolysins, hæmagglutinins, cytotoxines, hæmorrhagin, thrombokinase, and precipitin.[2] They differ not only in physiological action and chemical composition, but they show widely different labilities.

[1] Mitchell and Reichert, *Smithsonian Contributions*, 1886, No. 647; Cunningham, *Scientific Memoirs by the Medical Officers of the Army of India*, 1895, Part IX, and 1898, Part XI; Kanthack, *Report of Medical Officers of Local Government Board*, London, 1895–1896; Stephens and Myers, *Journ. of Path. and Bact.*, 1899–1900, xi, 415; Stephens, *idem*, p. 273; Flexner and Noguchi, *Journ. of Exp. Med.*, 1902, vi, 277; *Journ. of Path. and Bact.*, 1905, x, 111; *Univ. of Penna. Med. Bulletin*, 1902, xv, 360; Noguchi, *Journ. of Exp. Med.* 1905, vii, 191; Mitchell and Stewart, *Mem. of National Acad. of Sciences*, 1898, viii; Kyes, *Berl. klin. Woch.*, 1903, xl, 956, 982; Kyes and Sachs, *idem*, 1903, xl, 21, 57, 82; Lamb, *Indian Med. Gazette*, 1901, xxxvi, 443; Martin, C. J., *Proc. Roy. Soc. of N. S. Wales*, 1892, xxvi, 240.

[2] This is the globulin and hæmoglobin precipitating body described by me. *Journ. of Exp. Med.*, 1905, vii, 191.

If the variation in resistance of these principles to moist heat is taken the order will be about as follows: neurotoxin resists brief boiling; hæmolysin is destroyed at 135° C., hæmagglutinin at 75° to 80°C.; hæmorrhagins, cytolysins, and thrombokinase at 75° C.; precipitin at 96° to 100° C. Now since the venoms of different species and orders of snakes vary according to the prevalence of one or the other class of toxic constituents, the ease with which they succumb to heating depends on the nature of the predominant principles. Hence rattlesnake venom in which hæmorrhagin and possibly other locally acting non-heat resisting poisons are predominant, and daboia venom in which much thrombokinase is contained, are easily diminished in activity by heating to 75° C., at which temperature cobra venom suffers little change in toxicity.

The venoms of the cobra, *Crotalus adamanteus*, and *Daboia Russellii* were exposed to the action of eosin and erythrosin. These anilines were chosen because of their pronounced action upon ferments and toxins.

Action upon the Hæmolytic Principle.—The venoms were employed in the following strengths: daboia, 0.1 per cent.; cobra and rattlesnake, 0.4 per cent. The dyes were used in 0.25 per cent. solution. Four parts of the venom solution were mixed with one part of the aniline solution. Hence they contained 0.05 per cent. of the dye. The mixtures were divided into two parts, one of which was kept in the dark while the other was exposed to sunlight for thirty hours. The controls consisted of venom solutions of the same strength in salt solutions kept under identical conditions as the dye mixture. Dog's blood in 3 per cent. suspension was employed. The total volume in each tube was two cubic centimeters; the reading was made after two hours at 37° C. and overnight at room temperature.

TABLE I.

COBRA HÆMOLYSIN.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.1	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.05	"	"	"	Almost C.H.	"	"
0.02	"	"	"	"	"	"
0.01	"	"	"	Moderate H.	"	Almost C.H.
0.005	"	Almost C.H.	Almost C.H.	Slight H.	"	"
0.002	Moderate H.	Moderate H.	Moderate H.	Trace H.	Moderate H.	Moderate H.
0.001	"	"	"	No. H.	"	"
0.0005	"	"	"	"	"	"
0.0002	Slight H.	Slight H.	Slight H.	"	Slight H.	Slight H.
0.0001	Trace H.	Trace H.	Trace H.	"	Trace H.	Trace
0.00005	"	"	"	"	"	"
0.00002	"	"	"	"	"	"
0.00001	"	"	"	"	"	"
0.000005	"	"	"	"	"	"
0.000002	No. H.	No. H.	No. H.	"	No. H.	No. H.
0.000001	"	"	"	"	"	"
Control	"	"	"	"	"	"

A glance at Table I shows that the hæmolysin of cobra venom is reduced in activity very little by exposure to sunlight in the presence of eosin and not at all in the absence of the dye or in the presence of erythrosin. The hæmolysin is, therefore, resistant to the photodynamic action of these two fluorescent bodies. In view of this result it was to be expected that cobra-lecithid prepared by Kyes's method should remain unaffected, a fact which points not only to the relative resistance of the hæmolysin but also to the stability of the compound. A similar series of tests with Crotalus venom gave different results.

The hæmolytic power of Crotalus venom is reduced by almost 200 times the original strength when measured by absence of hæmolysis, and by almost forty times when measured by slight hæmolysis. The dyes are without influence in the dark and exposure to direct sunlight for the period of the experiment does not injure the hæmolysin in the colorless solution.

TABLE II.

CROTALUS HÆMOLYSIN.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.2	C.H.	C.H.	C.H.	Almost C.H.	C.H.	Almost C.H.
0.1	"	"	"	Moderate H.	"	Moderate H.
0.05	Almost C.H.	Almost C.H.	Almost C.H.	"	Almost C.H.	"
0.02	Much H.	Much H.	Much H.	Slight H.	Much H.	Slight H.
0.01	Moderate H.	Moderate H.	Moderate H.	Trace H.	Moderate H.	Trace H.
0.005	"	"	"	No. H.	"	"
0.002	"	"	"	"	"	No. H.
0.001	"	"	"	"	"	"
0.0005	Slight H.	Slight H.	Slight H.	"	Slight H.	"
0.0002	"	"	"	"	"	"
0.0001	"	"	"	"	"	"
0.00005	Trace H.	Trace H.	Trace H.	"	Trace H.	"
0.00002	"	"	"	"	"	"
0.00001	No H.	No. H.	No. H.	"	No. H.	"
0.000005	"	"	"	"	"	"
Control	"	"	"	"	"	"

Cobra and Crotalus hæmolysins exhibit, therefore, a wide difference as regards their susceptibility to injury by eosin and erythrosin. This difference is in keeping with the labilities which they show in general. It is, therefore, of interest to ascertain whether the hæmolysin of daboia venom is to be placed with the former or the latter in its reaction to these substances. Table III which gives the results of the test with daboia venom shows it to occupy an intermediate position since its loss of power fluctuates between $\frac{1}{5}$ and $\frac{1}{10}$ according to the level of hæmolysis at which it is measured. With both rattlesnake and daboia venom eosin appears to be a little more active than erythrosin, but the differences are slight.

Effects on the General Toxicity.—The three venoms—cobra, Crotalus, and daboia—used in testing the effect of eosin and erythrosin upon the general venom toxicities sufficed to bring out the relative degrees of resistance of the neurotoxic, hæmorrhagic, and blood-coagulating principles. The toxic action of cobra venom being due mainly to the first principle, any marked reduction in toxicity which might take place would be ascribable to changes in that substance, and the same reasoning is applicable to hæmorrhagin as represented in rattlesnake venom and coagulin in daboia venom.

TABLE III.

DABOIA HÆMOLYSIN.

Venom solution in c. c	Control		Eosin		Erythrosin.	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.3	C.H	C.H.	C.H.	C.H.	C.H.	C.H.
0.2	"	"	"	"	"	"
0.1	"	"	"	"	"	"
0.05	"	"	"	"	"	"
0.02	"	"	"	"	"	"
0.01	"	"	"	Almost C.H.	"	"
0.005	"	"	"	"	"	Almost C.H.
0.002	Almost C.H.	Almost C.H.	Almost C.H.	Moderate H.	Almost C.H.	Much H.
0.001	"	"	"	"	"	Moderate H.
0.0005	"	"	"	Slight H.	"	Slight H.
0.0002	Much H.	Much H.	Moderate H.	"	Much H.	"
0.0001	"	"	"	Trace H.	"	Trace H.
0.00005	Slight H.	Slight H.	Slight H.	No. H.	Slight H.	"
0.00002	"	"	"	"	"	No. H.
0.00001	"	"	Trace H.	"	"	"
0.000005	No. H.	No. H.	No. H.	"	No. H.	"
0.000002	"	"	"	"	"	"
0.000001	"	"	"	"	"	"
Control	"	"	"	"	"	"

The technique of the tests is simple. Stock solutions of venom are mixed with the dyes in solutions. The mixtures are divided into two parts one of which is exposed and the other kept in the dark. A simple solution of the same strength without any admixture is treated in the same manner—one half being exposed and the other placed in a dark chamber. The exposure to sunlight was, as a rule, for thirty hours.

Cobra Venom—0.2 per cent., stock solution 4 parts; 0.25 per cent., eosin or erythosin 1 part. All solutions made in 0.9 per cent. salt. Exposure 30 hours. Toxicity tested in guinea-pigs weighing 350 grams. Intraperitoneal injections.

TABLE IV.

COBRA VENOM AND GUINEA-PIGS.

Venom in grm.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.001	+ 1 h. 25 m.			+1 h. 30 m.		+ 45 m.
0.00075	+ 4 h. 50 m.					
0.0005	+ 2 h. 48 m.			+ 3 h.		+ 4 h. 10 m.
0.0003	+ 3 h. 34 m.					
0.00025	+ 6 h.					
0.0002	+ 12 h.	+ 18 h.	+ 12 h.	+ 24 h.	+ 18 h.	+24 h.
0.00015	+ 18 h.	+ 36 h.	+ 2 days	+ 2 days	+ 38 h.	+40 h.
0.0001	℥	℥	℥	℥	℥	℥
0.000075	℥	℥	℥	℥	℥	℥
0.00005	℥	℥				

+ =death. ℥=Survived.

The conclusion to be drawn from this series of tests is that the two anilines employed are without marked effects in diminishing the toxicity of cobra venom for guinea-pigs. On the other hand, with rabbits, a slight reduction in toxicity after the eosin treatment is apparent.

TABLE V.

COBRA VENOM AND RABBITS.

Rabbit No. 1. Venom 0.002 gram. Death in 20 minutes.
Rabbit No. 2. Venom 0.002 gram + eosin 0.05% (Dark). Death in 20 minutes.
Rabbit No. 3. Venom 0.002 gram + eosin 0.05% (Sunlight). Slightly ill; recovered.
Rabbit No. 4. Venom 0.003 gram + eosin as before (Sunlight). Death in 80 minutes.
Rabbit No. 5. Venom 0.004 gram + eosin as before (Sunlight). Death in 15 minutes.
Rabbit No. 6. Venom 0.008 gram + eosin as before (Sunlight). Death in 60 minutes.

The reduction in toxicity as measured on rabbits probably does not exceed $\frac{1}{3}$ and may be less, since the individual variation in susceptibility of the animals may affect the results. That such a factor operates at times is to be seen by comparing the results with Rabbits Nos. 5 and 6.

Crotalus Venom.—The preponderance of hæmorrhagin and the small quantity of neurotoxin contained in rattlesnake venom make it peculiarily suitable for tests of relative stability of these two classes of bodies. The symptoms and lesions of poisoning by rattlesnake venom can readily be interpreted. Hence any marked reduction in toxicity can be accounted for by the destruction of one or both of the chief toxic principles.

The venom was in 0.4 per cent. and the eosin and erythrosin in 0.25 per cent. solutions. The details of the method were the same as with cobra venom. Guinea-pigs of 250 grams were used for the tests, the injections being made into the peritoneum.

TABLE VI.

CROTALUS VENOM AND GUINEA-PIGS.

Venom in grm.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.03				+ 5 days*		+ 4 days*
0.024				+15 days*		℥
0.018				No symptoms†		No symptoms†
0.012				"		"
0.008				"		"
0.004				"		"
0.001	+ 6 h.‡	+ 5 h. 40 m.‡	+ 8 h.‡	"	+10 h.‡	"
0.0008	+ 8 h.	+12 h.			+15 h.	
0.0006	+10 h.	+24 h.	+12 h.		+10 h. 50 m.	
0.0005	+24 h.	+ 3 days	+ 24 h.		+24 h.	
0.0004	℥	℥	℥		℥	
0.0003	℥	℥	℥		℥	

+ = Death.
‡ = Very marked hæmorrhage in the peritoneal cavity, viscera, and muscles.
* = Died of emaciation, no hæmorrhage.
† = Killed with chloroform and the peritoneal cavity examined after 48 hours; no hæmorrhage.
℥ = Survived.

From this table it is seen that it is the hæmorrhagic principle that is destroyed by the dyes after exposure to sunlight. However, the neurotoxin would also appear not to be left wholly intact; but to what change in action of the venom the marasmic condition which developed in certain of the pigs is due is not known. It is well known that many of the bacteria and certain toxins cause in quantities below the lethal dose a state of malnutrition to which the animal may eventually succumb. The remote effects of the modified venom may be compared to this action of bacteria, ricin, and other toxins.

The time limit of thirty hours, while insufficient to reduce markedly the toxicity of cobra venom, is greater than is necessary to reduce considerably the toxicity of rattlesnake venom. A series of tests was made with the latter venom, which was exposed with eosin for eight hours to sunlight. The result with guinea-pigs of 250 grams each was as follows:

0.002 gram. Survived.
0.0032 " "
0.0048 " "
0.0064 " Death after 5 days. Slight hæmorrhage.
0.008 " Sick for ½ day; chloroformed after 36 hours; slight hæmorrhage.

The action of eosin in the light upon Crotalus venom is quite rapid. In eight hours about 9.6m. l. d. and about 16 m. hg. d.[3] of the venom were completely destroyed under the conditions of the experiment.

Daboia Venom.—While this venom is rich in hæmolytic and cytolytic principles its chief immediate peculiarity of action results from the thromobokinase which it contains. Whether neurotoxin is present in any but minimal quantities is still undecided. My experiments with eosin and erythrosin were directed against the blood-coagulating constituent. The venom was used in a proportion of 4 parts of 0.2 per cent. and the dyes in 1 part of 0.25 per cent. solutions. The methods employed were identical with those already given. The time of exposure was thirty hours; rabbits weighing 1500 grams were injected intravenously.

TABLE VII.

DABOIA VENOM AND RABBITS.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.03				+ 5 days*		+ 10 days*
0.02				⅔		⅔
0.015				+ 5 days*		No sympt.
0.01				⅔		"
0.008				No sympt.		"
0.006				"		"
0.004				"		
0.003				"		
0.0004	+ 5 min. ×	+ 2 min. ×	+ 6 min. ×		+ 3 min. ×	
0.0004	+ 4½ min. ×	+ 7 min. ×	+ 5 min. ×		+ 1½ min. ×	
0.0004	+ 7 min. ×	+ 6 min. ×				

+ = Death. ⅔ = Survived after some illness.
× = Intravascular thrombosis.
* = Died of marasmus.

Since these experiments (Table VII) show that the clotting principle of daboia venom is completely destroyed by the fluorescent dyes employed in the experiments, and since they also show that by this treatment the general toxicity of the venom may be considerably reduced, a second series of experiments was made to ascertain roughly the rapidity with which the toxicity is diminished by means of eosin. Rabbits of 1500 grams weight were employed.

[3] Minimal hæmorrhagic dose.

TABLE VIII.

DABOIA VENOM AND RABBITS.

Time of exposure	Dose of venom	Results
30 minutes	0.0004 gram.	No symptoms
" "	0.0006 "	+ 5 minutes
" "	0.0008	+ 5 minutes
3 hours	0.0016 "	No symptoms
" "	0.0024 "	+ 7 minutes
6 hours	0.032 "	No symptoms
" "	0.004 "	" "
" "	0.006 "	Sick; survived
8 hours	0.0016 "	No symptoms
" "	0.0032 "	" "
" "	0.0064 "	" "
12 hours	0.008 "	No symptoms
30 hours	0.01 "	No symptoms

These experiments show that the principle causing intravascular clotting of the blood is quickly and readily destroyed by eosin in the light and that 75 m. l. d. of eosinized venom may not contain one m. l. d. of the thrombokinase. The death of the rabbit, which took place after injection of a large amount of the modified poison was due probably to the combined action of the hæmolytic and neurotoxic residues.

Action upon the Red Corpuscle-Protecting Principle.—In a previous publication [4] I described a new property of venom, namely, its capacity to unite with the globulin constituents of blood serum to form an insoluble compound which is precipitated, and with the globin of hæmoglobin which it also renders insoluble in water and weak saline solution. Because of its action upon hæmoglobin strong solutions of cobra venom protect blood corpuscles from water, and even from energetic chemical hæmolysis. Experiments were made with eosin and erythrosin to determine whether this property of cobra venom is destroyed by these agents under the influence of light.

[4] *Jour. of Exper. Med.*, 1905, vii, 191.

The cobra venom was used in 20 per cent. solution, of which equal parts and 0.1 per cent. solutions of the dyes were mixed. One part of the mixture was exposed to sunlight for 12 hours and the other kept in the dark as a control. The solutions for precipitation consisted of (1) washed rat's corpuscles laked in 9 parts of water and centrifugated to remove the stroma, (2) aqueous solutions of Merck's hæmoglobin, and (3) rat's serum diluted with water. The tests were made with 0.8 c.c. of the solutions enumerated and 0.2 c.c. of the venom-dye mixture. The protective action on the corpuscles was tested by adding 0.3 c.c. of washed rat's corpuscles to 0.6 c.c. of the venom-dye mixture. The results are given in Table IX.

TABLE IX.

		Control (no dye)		Eosin.		Erythrosin.	
c. c.	Control (no venom)	20% Cobra venom 1 part Distilled water 1 part.		20% Cobra Venom 1 part 0.1% Eosin 1 part.		20% Cobra venom 1 part 0.1% Erythrosin 1 part.	
		Dark	Exposed	Dark	Exposed	Dark	Exposed
Blood solution 0.8 The fluid tested 0.2	Clear	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (coarser) (18 hours)	Voluminous precipitate (coarser) (18 hours)
2% aqueous solution of hæmoglobin (Merck) 0.8 The fluid tested 0.2	Clear	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)
Rat's serum 0.2 H_2O 0.6 The fluid tested 0.2	Clear	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)
Rat's blood corpuscles (washed thrice) 0.03 The fluid tested 0.6	No hæmolysis	Protected	Protected	Protected	Protected	Protected	Protected

The result is definite. The globulin precipitating and corpuscle-protecting principle of venom does not undergo marked change in sunlight in the presence of eosin and erythrosin after an exposure of 12 hours. If erythrosin in a strength equal to one per cent. of the mixture is present, the venom-hæmoglobin precipitation does not occur, and rat's corpuscles are prevented from protection against hæmolysis. In respect to this action of erythrosin, it should be added that Sacharoff and Sachs state that in solutions of high concentration this dye is itself hæmolytic.

SUMMARY.

Since the hæmolysins of the several venoms respond differently

to photodynamic action, they may be regarded as possessing different chemical constitutions. As regards stability, cobra hæmolysin ranks first, daboia second, and Crotalus third.

The toxicity of all the venoms is more or less diminished by eosin and erythrosin in sunlight. This reduction in toxicity depends upon chemical changes, of more or less profound nature, taking place in certain of the active principles of the venom. The more stabile the predominant active principles the less the reduction in toxicity, and vice versa. Venom-neurotoxins are highly resistant to photodynamic action, venom-hæmolysins are less resistant, while the hæmorrhagin and thrombokinase of Crotalus and daboia venoms exhibit weak powers of resistance to their action. Hence it follows that while cobra venom remained almost unaltered, rattlesnake and daboia venoms were greatly reduced in toxicity when mixed with the fluorescent dyes and exposed to sunlight.

There is an interesting parallel between the action of eosin and erythrosin upon the different venoms and their reactions to other injurious agencies. For example, the hæmolysins of cobra and daboia venoms are more heat resistant than the hæmolysin of Crotalus venom, and the former are less injured by the dyes than the latter. The neurotoxin of the former venoms is also more heat stabile than that of the rattlesnake, and the same relative degree of resistance holds for this substance and the anilines. Just as the hæmorrhagin of rattlesnake venom and the thrombokinase of daboia venom are destroyed by a temperature of 75° C., so are they readily inactivated by the photo dynamic substances employed.

The globulin-precipitating and blood corpuscle-protecting principle of cobra venom is relatively thermostabile and in contradistinction to the immunity-precipitins it is also unaffected by eosin and erythrosin.

This study of the action of photodynamic substances upon snake venoms serves again to bring out the fact of their highly complex nature, and while enlarging somewhat the field in which photodynamic activity is known to operate, it also proves that this form of destructive activity is affected by the same con-

ditions of resistance as confront the action of the usual physical and chemical agents.

THE LITERATURE ON PHOTODYNAMIC ACTION.

In this brief review only the salient facts can be given. The literature on the biological effects of fluorescence has already grown into a considerable bulk. We shall begin with v. Tappeiner's (1) studies on the chinin derivatives. He proved that the toxic property of chinin resided in the chinolin nucleus and that the toxicity of this body was increased when methoxyl and methylene radicals were present in the side chains. V. Tappeiner further endeavored to increase the toxicity of chinolin by bringing about a further condensation with the benzol nucleus. The proof of the correctness of this view was brought by Grethe (2), who found that the dye phosphin exerted intense toxic action upon paramecium. Raab (3) noted that muriate of acridin in a 1 : 1000 dilution kills paramecium instantly; while weaker solutions acted with disturbing irregularity. The cause of this irregularity was finally traced to the influence of light. Thus in a solution of 1: 20,000 in the dark the protozoa are unaffected, while in diffuse sunlight they are destroyed in 60 minutes, and in direct sunlight in 6 minutes. Raab also ascertained that the action of all protoplasmic poisons is not intensified by light: morphine, phenylchinaldin, and strychnin are not modified in their effects by light. Raab discovered that the increased action of light depends upon fluorescence of the chemical agents. Minute studies have now been made of this property. Raab found that when sunlight passes through a fluorescent solution it is robbed of its power to set up fluorescence in a second solution. No intensification of toxic action is produced in the second solution by the filtered light. Fluorescent light itself is without toxic action upon infusoria; to obtain this effect the living organisms must be immersed in the fluorescent fluid.

We owe to v. Tappeiner the term "photodynamic action," which describes the toxic effects produced by fluorescent chemicals in the light. This activity does not depend upon the absorption of light rays, because some of the most active absorbing agents were shown by v. Tappeiner and Jodlbauer (4,5) not to have their toxicity increased by light. All fluorescent substances would seem to be able to exert photodynamic action; but there is much variation in the intensity of action among the different bodies themselves, and a further variation appears according to the substances—living cells, ferments, toxins—upon which the action is exerted. The relation of degree of fluorescence and intensity of photodynamic action is, according to v. Tappeiner and Jodlbauer (6) a reverse one. Strong solutions may act more energetically than weak ones, but a quantitative comparison shows that, as a rule, the weaker concentrations of the fluorescent body are the more active (7). When certain members of a chemical group do not possess fluorescence they tend to be devoid of photodynamic action. Many salts of fluorescein are photodynamically active; but the non-fluorescent salts—tetranitrofluorescein, phenolphthalin, hydrochinonphthalin—are inactive (8).

Besides the degree of fluorescence and concentration of the chemical body, the intensity of the light plays an effective part. Dreyer (9) found that the

infusorian Nassula was killed in 10 seconds under the combined influence of eyrthrosin and Finsen's light, while the Finsen light alone required 9 minutes to produce this effect. Dreyer and Neisser and Halberstaedter (10) viewed the erythrosin merely as a sensitizer and ranked it with neutral red, which they regarded as devoid of fluorescent power. More searching study has brought out the fact of neutral red's possession of feeble fluorescent properties; and v. Tappeiner (11) has been able to show further that sensitization of a photographic plate and photodynamic action are independent powers. Several active sensitizers—bisulphite alizarin blue, diazo black, glycin red, nigrosin, ethylene red—are non-fluorescent and also photodynamically inactive.

In the meantime Straub (12) observed the dissociation of potassium iodide in the light by eosin and chinin, and thus opened the way for the study of photodynamic action as a process of oxidation, proofs of which form of activity were supplied by Edlefsens' (13) studies which were confirmed by Jodlbauer and v. Tappeiner (14,15). The last-named investigators made a comparative study of the oxidative and biologically injurious properties of photodynamic substances and found that a parallel did not exist. The strongest oxidizing body is not the most effective photodynamic agent. Thus aesculin is an energetic oxidizer but has weak photodynamic power, while the biologically active phenosafranin and azocarmin are incapable even of dissociating iodide of potassium. Jodlbauer and v. Tappeiner also found that photodynamic action may exceptionally take place in fluids from which the free oxygen has been removed. Bie (16) believed the bactericidal action of light not to depend on oxidation. This part of the subject clearly calls for further study pending the setting up of conclusions.

A considerable number of unicellular organisms, toxins, ferments, and antitoxins have been studied in relation to photodynamic activity. The infusoria have been especially studied by Raab (17), v. Tappeiner (18), Jodlbauer and v. Tappeiner (19), and Ledoux-Lebard (20). They all show marked sensitiveness to this form of injury. Jacobson (21) has found that the ciliated tracheal epithelium of the frog is injured by acridin especially in the light. Sachs and Sacharoff (22) and Pfeiffer (23) have described the hæmolytic action of certain fluorescent dyes. Some of these are active in the dark the action being, however, intensified by light (24).

Bacteria are subject to photodynamic action as is shown by the observations of Dreyer (25), Raab, Jodlbauer and v. Tappeiner (26), Huber (27), and Bie (28). Huber found that the filtration of the light through ruby glass prevents the injurious action. Fungi are also susceptible to the action of certain of the anilines. Lichtwitz (29) found that the complements but not the hæmolytic immune bodies of normal and immune serums are destroyed by eosin in the light. And according to Fleischmann (30), eosin and light together so modify precipitin that it no longer causes a precipitate with its corresponding precipitable substance, although it is still capable of entering into chemical union with it.

The ferments show varying degrees of susceptibility to photodynamic activity. Diastase, invertin, papayotin (31), trypsin, and chymosin (32) are affected more or less strongly. Eosin and magdala red are among the most active anilines. Diastase is more resistant than invertin and papayotin. Many fluorescent,

photodynamic bodies do not act injuriously upon the ferments. According to Huber (33), rennet is little affected by simple exposure to light, while in the presence of eosin or erythrosin its coagulating action is much delayed. Katalase remains unaffected by any of the fluorescent bodies tested (34).

Bacterial and other toxins succumb to this form of activity (35). Diphtheria toxin is much reduced in power by eosin and light. Other fluorescent anilines are also effective on this poison. V. Tappeiner and Jodlbauer observed that guinea-pigs could be protected from one m. l. d. of diphtheria toxin by previous injection of eosin or methylene blue and daily exposure to the sun. Doses of 3 to 4 m. l. d. were uniformly fatal after this treatment. Huber (36) also found that diphtheria toxin is destroyed by eosin in the light. V. Tappeiner and Jodlbauer noted the modification or destruction of tetanus toxin by certain dyes. They found that after treatment 10 m. l. d. of the toxin did cause local tetanus, while 25 m. l. d. caused fatal tetanus. Huber noted the gradual deterioration of tetanolysin in the light and its rapid destruction in the presence of eosin. Flexner and Noguchi (27) showed that tetanolysin and tetanospasmin are destroyed *in vitro* by eosin, and rats can be protected from fatal tetanus infection by local applications of the dye. Ricin (37) is readily attacked. V. Tappeiner and Jodlbauer found that its agglutinin is totally destroyed by a large number of anilines, while the other toxic constituents are more resistant.

Mammals are not insusceptible to the action of these bodies. Acridin may cause abscesses in rabbits (5), and mice are subject to local necroses if after injection they are exposed to the light. A peculiar effect is the necrosis of the ears of mice, which follows the injection and exposure (38). Exophthalmus has been noted in rats injected with Rose bengale. After injection of eosin, erythrosin, and Rose bengale into the shaved skin of rabbits and exposure to sunlight, an area of necrosis with exfoliation of the tissue develops.

V. Tappeiner and Jesionek (39, 40) have treated with success patients suffering from a variety of parasitic and neoplastic conditions of the skin by applying eosin and exposing the parts to sunlight. Artificial light may be substituted for sunlight and injection into the tissues may sometimes take the place with advantage of superficial application of the dye.

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THE EFFECT OF EOSIN AND ERYTHROSIN UPON THE HÆMOLYTIC POWER OF SAPONIN.

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The chemical action of photodynamic substances is very wide. Not only do they cause alteration of the labile molecules of ferments and toxins, but they are definite and even intense protoplasmic poisons. Their action is, however, not limited to the complex structures and molecules of these bodies, but is capable of being exerted upon much simpler and, chemically speaking, more definite substances.

The more recent investigations of v. Tappeiner,[1] Jodlbauer, Straub,[2] Edlefsen,[3] and Sacharoff and Sachs,[4] all tend to show that fluorescent photodynamic chemicals act by promoting oxidation of the cells, toxins, etc. A wide variety of inorganic and organic well-defined chemical substances was proven to undergo oxidation in the presence of the photodynamic reagents. There were certain exceptions as, for example, dextrose and formaldehyde, which remained unaltered.

In view of these facts, and as a complement to my studies on snake venom, I tested the action of eosin and erythrosin upon the hæmolytic property of saponin. The following tables show that eosin and especially erythrosin act with considerable intensity upon saponin.

The saponin was Merck's, the dyes "eosin rein" and "erythro-

[1] Jodlbauer und v. Tappeiner.—Ueber die Beteiligung des Sauerstoffes be der Wirkung fluorescierender Stoffe. *Deutsch. Arch. f. klin. Medicin*, 1905, lxxxii, 520.

[2] Straub.—Ueber chemische Vorgänge bei der Einwirkung von Licht auf fluorescierende Substanzen und die Bedeutung dieser Vorgänge für die Giftwirkung. *Münch. med. Woch.*, 1904, li, 1093.

[3] Edlefsen—Experimenteller Beitrag zum Studium der fluorescierenden Stoffe. *Münch. med. Woch.*, 1904, li, 1585.

[4] Sacharoff und Sachs.—Ueber die hæmolytische Wirkung der photodynamischen Stoffe. *Münch. med. Woch.*, 1905, lii, 299.

sin pur." were Grübler's. Saponin was employed in 0.1 per cent., dissolved in 0.9 per cent., salt solution. The dyes were employed in concentrations of from 0.05 per cent. to 2.5 per cent. The mixtures of saponin and dye were divided into two portions, of which one was kept in the dark, the other being exposed to direct sunlight for twelve hours. Control solutions of saponin were treated in the same manner. Two kinds of corpuscles—from the dog and the rat—were used in 3 per cent. suspension. After four hours in the thermostat at 37° C. and eighteen hours at room temperature the readings were made.

TABLE I.

Saponin 0.1%; fluorescent anilines 0.05%; dog's corpuscles.

Saponin solution 0.1%	Control		Eosin 0.05%		Erythrosin 0.05%	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
1.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.
0.7	"	"	"	"	"	"
0.5	"	"	"	"	"	Much H.
0.4	"	"	"	"	"	Moderate
0.3	"	"	"	"	"	Slight H.
0.2	"	"	"	Much H.	"	None
0.15	"	"	Much H.	Slight H.	Much H.	"
0.1	Much H.	Much H.	Moderate	None	Moderate	"
0.07	Slight H.	Slight H.	Trace	"	Trace	"
0.05	None	None	None	"	None	"
0.04	"	"	"	"	"	"
0.03	"	"	"	"	"	"
0.02	"	"	"	"	"	"

TABLE II.

Saponin 0.1 %; eosin 0.05 %; rat's corpuscles (washed).

Saponin solution	Control		Eosin 0.05%	
	Dark	Exposed	Dark	Exposed
1.	C. H.	C. H.	C. H.	C. H.
0.7	"	"	"	"
0.5	"	"	"	"
0.4	"	"	"	Almost C. H.
0.3	"	"	"	Much H.
0.2	"	"	Much H.	Slight H.
0.15	"	"	Slight H.	None
0.1	Almost C. H.	Almost C. H.	Slight H.	"
0.07	Much H.	Much H.	None	"
0.05	Slight H.	Slight H.	"	"
0.04	None	None	"	"
0.03	"	"	"	"
0.02	"	"	"	"

The tables show that saponin alone, in the strength solutions employed, does not deteriorate quickly; and they also show that solutions of this substance have their hæmolytic power

considerably reduced when exposed to sunlight in the presence of minute quantities of eosin and erythrosin, and these fluorescent anilines exert a slightly reducing effect upon this property even in the dark. The maximal loss of hæmolytic power equalled in twelve hours about $\frac{1}{4}$ to $\frac{1}{8}$, and in seventy-two hours $\frac{1}{14}$ the total strength.

That concentration of the solutions plays a part in the experiment is indicated by the resistance which a one per cent. solution of saponin exhibited in the presence of eosin in strength of 2.5 per cent. The saponin was not in this case perceptibly reduced in hæmolytic strength after twelve hours' exposure to direct sunlight. If, however, a one per cent. saponin solution is mixed with 0.05 per cent. eosin it is reduced to one-fifth its hæmolytic strength after seventy-two hours' direct exposure to sunlight. V. Tappeiner noted that strong solutions of photodynamic substances may be inactive where weaker ones are effective.

The change in constitution which the saponin undergoes in the presence of eosin and erythrosin has not been established. It would seem, from certain indirect experiments which I have made, not to depend upon the usual splitting of the glucoside. While I did not determine the absolute absence of dextrose in the exposed mixtures, yet I can say that a quantity equal to 0.001 per cent. was not present. Beyond this limit the copper tests which I used were not reliable.

CONGENITAL CYSTIC KIDNEY AND LIVER WITH FAMILY TENDENCY.

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So much has already been written concerning congenital cystic kidney that an additional communication upon the subject may seem to demand an apology. The author's excuse is a recent opportunity to study two cases in which the lesion was so early that they seem to offer some indication as to the pathogenesis of the condition. The cases were both new-born infants delivered in the obstetrical service of Dr. J. Whitridge Williams in the Johns Hopkins Hospital. Some interest attaches to the fact that they were both children of the same mother, being the first and second children of Mary F., an apparently healthy negro who was sixteen years of age at the time of the delivery of her first child in April, 1904. Her first confinement occurred on April 14, 1904, when a perfectly normal labor resulted in the birth of a male child 44 centimeters in length and weighing 2026 grams. The labor was clinically estimated to have occurred early in the ninth month of pregnancy. The child was placed in the incubator and seemingly did well, gaining in weight after the initial loss, but it died suddenly on the eleventh day.

The second child, a girl, was born on March 21, 1905, eleven and a half months after the birth of the first child. The child was also born prematurely, apparently toward the end of the eighth month of pregnancy. Labor was normal. The child was 42.5 centimeters in length and weighed 2005 grams at birth. It did not do well after birth but lost gradually in weight up to the time of its death when it weighed 1380 grams.

The abbreviated post-mortem protocols of both children will be found appended to this article. For the sake of convenience in description the children will be designated child "A" and child "B."

This family tendency has been previously noted a number of times, not however, in the negro race, and I emphasize it here not only to indicate the part inheritance plays in the condition but also to show that the cases here reported are related in this feature to a considerable group of cases of cystic kidney. Dunger[1] in his admirable review of the subject of congenital cystic kidney has collected eleven cases and added one of his own observation, in which the lesion has occurred in two or more members in the same generation or in two generations of a single family. To these I am able to add several others from literature in addition to the present case.

Schupmann[2] is often credited with having reported the first case showing family tendency, but a reference to his article shows, as Dunger suggests, that he reports not the occurrence of congenital cystic kidney in three children but in the third child of a mother who had given birth to two malformed children previously. This child showed in addition to the kidney lesion hydrocephalus with encephalocœle and a bicornuate uterus and double vagina. Luzzatto[3] includes Adamkiewicz[4] among those who report cases of family tendency, but I have been unable to gain access to his thesis. Virchow[5] in 1855 published the notes of a family in which the mother gave birth successively to three premature children. A post-mortem examination on the third showed bilateral cystic kidneys as the cause of the abdominal swelling. A subsequent child showed a similar condition at the autopsy, and there is little doubt that the same condition existed in the other two children which were not examined. The mother gave birth to four other children, who lived, although one died at the age of ten months as the result of acute hydrocephalus. In 1867 Wolff[6] reported a case of bilateral cystic kidney associated with hydrocephalus in the male child of a woman who had given birth to a female child, previously recorded, with similar lesion of the kidneys eleven months before. The woman had previously borne healthy children. In 1869 Brückner[7] reported two children of the same mother, the third and seventh child, with cystic kidneys and other malformations. The first of these two children showed six fingers on the right hand and seven on the left. The head was set far backward almost on the buttocks and there was also hydrocephalus with hernia of the brain. The second child showed six digits on both hands and both feet and also had

[1] *Ziegler's Beiträge*, 1904, xxxv, 445.
[2] *Organ f. d. ges. Heilkunde*, 1842, ii, 135.
[3] *La degenerazione cistica dei reni*, Venice, 1900.
[4] Inaugural Dissertation, Berlin, 1843.
[5] *Verh. d. Phys. Med. Gesellsch.*, Würzburg 1855, v, 447.
[6] *Berlin klin. Woch.*, 1867, iv, 480
[7] *Virchow's Archiv*, 1869, xlvi, 503

hydrocephalus with encephalocœle and atresia of the hymen. In 1894 Singer[8] described a family in which the first (male), fourth (male), seventh (female), tenth (female), and thirteenth (male) children showed the lesion of congenital cystic kidneys, the tumor masses causing great difficulty in delivery, resulting in the death of the children at birth. Only three of these cases were examined post-mortem, but again the presence of tumor masses in the region of the kidney leaves no doubt as to the existence of the lesion in the two unexamined cases. Carbonnel[9] has found the lesion in the kidneys of twins. Höhne[10] in 1896 reported the removal at operation of a cystic right kidney from a girl of 20 years whose mother, dying at the age of 49 years, had shown bilateral cystic kidneys, cystic liver, and cysts in the ovaries. Another child of the same woman had died at the age of 9 weeks of some kidney disease, the author suggests possibly also cystic disease. Steiner[11] in 1899 recorded two interesting families. In the first a man aged 52 died showing bilateral cystic kidneys and cystic liver. His sister also showed the condition of cystic kidneys, and his son of 10 years showed at autopsy a cystic right kidney, while several smaller children were also suspected to have the disease because of albuminuria and palpable nodular kidneys. In the second family the diagnosis of the condition of cystic kidney had been established in a man aged 46, and in a sister aged 38, while it had been demonstrated post-mortem in another sister dying at 42 years of age. In the ten-year-old son of the man a cystic right kidney had also been diagnosed. David and Jacobsohn[12] in 1900 found bilateral cystic kidneys at the autopsy on a woman 50 years of age whose sister had shown post-mortem a similar lesion. The mother of these two women who had died previously presented symptoms suggesting that she had this disease, though it was not actually determined by post-mortem examination. These authors quote Lauenstein's observation of cystic kidney in mother and daughter, as the first to denote a family tendency, but I have been unable to find the original publication. In 1901 Beck[13] reported an operation on a woman of 55 years at which a cystic left kidney was removed. After death 11 days later the other kidney was found extensively diseased. Two sisters of the patient had died previously from the same disease, one at 40 years after an operation, and the other at 54 years. Carrez[14] records the establishment of the clinical diagnosis of bilateral cystic kidney in a woman of 48, as a result of finding at operation a large cystic left kidney in her daughter of 27. Morris[15] records the following two cases. Two children of one mother died of congenital cystic kidney, one at 6.5 months, the other at 3.5 months. Of four other children in the family 3 showed other congenital defects and one was born dead. The father of these children was the only survivor of 13 children,

[8] Inaugural Dissertation. Greifswald. 1894.
[9] Quoted by Couvelaire, *Annales de gyn. et obst.*, 1899, lii, 453.
[10] *Deutsch. med. Woch.* 1896, xxii, 757.
[11] *Deutsch med. Woch.*, 1899, xxv, 667.
[12] *Charité-Annalen*, 1900, xxv, 16.
[13] *Annals of Surgery*, 1901, xxxiii, 147.
[14] Thesis, Lyon, 1901.
[15] *Surgical Diseases of the Kidney and Ureter*, 1901, i, 656.

eleven of whom had died at birth and the other at five months. Morris quotes also the report of Bar of three successive children of the same mother, who had died of the disease. Osler[16] in 1902 reported the establishment of the diagnosis of bilateral cystic kidney in a man aged 39, whose mother had shown a similar condition at autopsy. Meyer[17] reported in 1903 the autopsies on two brothers, one 8 months old, the other two years, who had died of diphtheria. In one of these there was present an atrophic cystic kidney on the right side, and in the other a similar lesion on the left. Dunger[18] reports observing a mother aged 64 years and daughter aged 26 years in both of whom bilateral cystic kidney and cystic liver occurred. Both died of cerebral hæmorrhage.

In this list it may be noted that a number of the cases showed, in addition to the cystic kidneys, other lesions in the nature of congenital malformations, and such is the frequency of this association that it may be considered almost the rule. The list of associated malformations is long and varied. It is headed apparently by hydrocephalus, although polydactylism is of frequent occurrence. It includes varied lesions to be attributed to imperfections in development in the complicated embryonic folds of the face and perineum, such as hairlip, hypospadias, atresia of the vagina, vesico-rectal fistula, rudimentary condition of the external genitalia. Malformations of the internal genitalia are also noted. In a few cases there have been defects in the cardio-vascular system, among them absence of the ductus Botalli, defects in the interventricular septum and of the aortic valves. In one case there was situs transversus of the thoracic viscera; in another absence of the spleen.

The two cases here reported, however, showed no such gross defects or malformations. Both children though small and prematurely delivered were well-formed externally and their organs were apparently normal, grossly. On microscopical examination, however, there was found in addition to the cystic condition of the kidney, the early stage of a similar cystic condition in the liver, and in one case also in the pancreas. This brings the cases into a fairly large group of cystic kidneys. Lejars[19] collected from literature 60 cases of cystic kidney and of these 28.3 per cent. showed also the cystic

[16] *American Medicine*, 1902, iii, 951.
[17] *Virchow's Archiv*, 1903, clxxiii, 209.
[18] Loc. cit.
[19] Thesis, Paris, 1888.

lesion in the liver. Luzzatto [20] in 1900 in a larger series of cases found 43 cases or 19.11 per cent. with the associated lesion in the two organs. This association seems important in considering the pathogenesis of the disease and will be discussed later. Turning to the kidneys themselves I shall first describe those of the second child, child "B" as they showed an earlier stage of the lesion than was shown by those of child "A."

The kidneys were normally placed in the body cavity and approximately of normal size, perhaps slightly enlarged. The ureters, bladder, urethra, and genitalia were normal. The ureters allowed the passage of a small probe with ease. The kidneys showed nothing abnormal until they were incised, when a considerable amount of urinous fluid poured out and it was found that a number of small cysts had been incised. The larger of these were about 2 or 3 mm. in diameter while there were many others scarcely visible. The cysts appeared to the naked eye to be confined almost exclusively to the medulla of the kidney, with here and there a visible cyst in the cortex. The different medullary pyramids did not appear equally affected, some of them being almost free from visible cavities. The two kidneys were affected and apparently almost equally so.

The tissues were fixed in Zenker's fluid and six sets of serial sections cut in paraffin from various parts of the organs, including a series from the part most affected as well as one from the area apparently freest from lesions. The sections showed that the kidneys in general were well formed. The cortical striation was well marked, with a normal relation between medullary rays and labyrinth, and the cortex was of normal width, measured by the number of rows of glomeruli between the capsule and the medulla which the study of numerous infantile kidneys has shown to be as a general average ten. The glomeruli were well formed and appeared normal with very scattered exceptions. In a few sections were found one or two glomeruli with a dilated capsular space. The tubules of the secreting type were also normal in appearance having a high granular epithelium and a small undilated lumen. In the medullary rays the loops of Henle appeared shorter than normal and in many places did not reach to the boundary zone. There were few cysts in the cortex until one came to a portion of the labyrinth bounding on the medulla. Here there were many elongated and oval cystic cavities lined by a fairly high cubical epithelium. By following out series of sections these could easily be found to be continuous at one end with tubules which entered the medullary rays and followed a course toward the medulla. They often joined other tubules of the collecting type. These tubules were unconnected with the loops of Henle, and were quite evidently collecting tubules, and the cysts thus corresponded to the portions of the tubular system known as the junctional tubules. The connections at the distal end of the cysts were more difficult to trace, but in several places they were found to pass over into tubules with the secreting type of epithelium.

[20] Loc. cit.

In the medulla the cystic formation reached its maximum. In many places there was scarcely a tubule found with a normal lumen. All were dilated. The cysts were larger than those of the cortex and were variously shaped, tending to be circular and were lined by high cubical epithelium. On the cortical side the cysts opened into several finger-like tubular projections which tapered rather abruptly so that in the boundary zone they were of the width of normal ducts and from here were continued as collecting tubules into the medullary rays. Toward the papilla the cysts became somewhat contracted and in favorable sections could be seen opening freely at the apex of the papilla, into the pelvis. There was no obstruction to the lumen of the ducts at the papilla. In very many places mitotic figures could be found in the lining epithelial cells of the cysts, indicating an active proliferation rather than any compression. There was, however, no evidence of numerical increase in the ducts. Intracystic papillary growths were not made out.

In the kidneys of child "B" the interstitial tissue of the medulla was not increased in amount. The walls of the adjacent cysts lay almost against each other, separated by only a few cells and strands of a rather embryonic connective tissue. The same condition held true for the cortex. In only one or two places was there a slight patchy increase in connective tissue and it was in relation to these patches that the few dilated glomeruli were found.

The sections from the part of the kidney which grossly appeared almost unaffected showed a condition similar to, but of less degree than, that in the other parts. There were almost as many cysts in both cortex and medulla, but they were smaller, resembling more a simple dilatation of the affected tubules.

There is clinical evidence that the kidney functionated, if not normally at least approximately so. The child lived 19 days and apparently passed a normal amount of urine into its napkins. Death was the result of acute gastro-intestinal infection. The sections showed also that the kidney was active and that there was no obstruction to the passage of urine through the tubules. In some of the cysts and tubules there were uric-acid deposits but not in such amount as to cause any apparent obstruction. In some of the tubules of the cortex there were hyaline casts and in one or two places such narrow casts were found projecting into the cortical cysts, demonstrating the continuity of these cysts with the tubules of the secreting type. There was precipitated albumin in some of the glomerular spaces and in the tubules, but no red blood cells were demonstrated.

The kidneys of child "A" were in general like those of child "B." They were about normal in size though the left kidney seemed slightly enlarged. This enlargement was found to be due especially to one fœtal lobule which projected above the others as a rounded pale mass. On section this was found to be much more cystic than the rest of the kidney. The cysts were large,

measuring in places 5 mm. in diameter. The tissue between them was pale and fibrous in appearance. In several places in the medulla there were small hæmorrhages, and uric-acid deposits were made out in the form of bright yellow dots and streaks. The microscopical sections showed also a marked similarity to those already described. There was, however, one important difference, and that was a new growth of connective tissue, most marked in the medulla but extending also into the portions of the cortex implicated. This connective tissue was quite richly cellular, but a Mallory connective-tissue stain showed the presence of abundant fibers which had a tendency to take a concentric arrangement about the cystic tubules. In this kidney the greater part of the cystic development was in the medulla, though the cortex was also implicated, and the cortical cysts occupied a position similar to those in the other case, in the portion of the labyrinth nearest the boundary zone. There were, in this kidney also, hæmorrhages into the interstitial tissue of the medulla.

Before treating of the pathogenesis of the renal lesion, I shall describe briefly the lesions of the other organs in so far as they have a bearing on the subject. The liver merits most attention and in the lesions of this organ again child "B" showed an earlier stage than child "A."

Grossly the liver of child "B" appeared slightly smaller than normal for an infant of that age. Superficially it was normal in appearance, the surface being smooth throughout, and the lobulation distinct. The gall bladder and bile ducts were normal. The cut surface, however, had a striking appearance and one that is apparently characteristic of this condition in its early stages. The parenchyma was quite normal in appearance, but everywhere throughout the organ the portal systems were sharply outlined as grayish translucent somewhat branched areas, resembling rather closely an oak leaf in shape. These spaces were evidently much wider than normal. There was a similar increase of connective tissue about the entering portal vessels at the hilum of the organ. The microscopical sections showed that the cause for the increase in the size of the portal spaces was a marked increase in the size and number of the ducts in them, with a slight increase in the connective tissue. This change was uniform throughout the organ. The ducts were lined by a high columnar epithelium, and were free from content other than a finely granular eosin-staining precipitate. These ducts were tortuous, were much branched, and were irregularly dilated into cyst-like spaces. In many places they could be traced to the outer limits of the lobules, where they ended in columns of liver cells of the secreting type. In other places they entered the lobules accompanied by a connective-tissue sheath, and in many places cross-sections of a duct were found, with a similar high epithelium with clear protoplasm, well inside the lobule and even close beside the central vein. The ducts with their cyst-like dilatations in the portal spaces were surrounded by an increased amount of connective tissue which was richly cellular and contained many cells of the lymphoid and plasma-cell type, as well as many eosinophiles. As stated, the condition prevailed throughout the liver, as shown by sections from various

parts of the organ. The picture offered by the liver is identical with that drawn to illustrate a case of Borst.[21]

The liver of child "A" was almost identical in gross and microscopical appearances with that of child B, the only difference being a more marked increase in the connective tissue, a difference already noted as existing between the kidneys of the two. The connective tissue in this child's liver was also less cellular and more fibrous and showed a tendency to encapsulate the ducts with concentric layers of fibers.

In the pancreas of child "B" there was found a similar hypertrophic condition of the duct epithelium. The larger ducts showed a much-folded involuted epithelium, and there were more numerous branching ducts in the spaces about the major ducts. Mitotic figures were found in the duct epithelium. The cells of the parenchyma were not so high nor so granular as those of a normal pancreas, and resembled more the duct epithelium. There was some increase in the connective tissue of the organ especially near the larger ducts. It was richly cellular and resembled the tissue about the ducts in the liver. This condition was less marked in the pancreas of child "A."

Section of the duodenum of child "B" showed a definite tendency to hyperplasia of the epithelium, though one hesitates to assign to it any important connection with the lesions of the organs described. The crypts of Lieberkühn were very deep and wide with a high coarse-appearing epithelium of an indifferent type. Some of the crypts were indeed cystic.

In the lungs of both children the terminal bronchi were extremely dilated, but there was no sacculation and the lesion was not apparently of the nature of a congenital bronchiectasis, but of a compensatory nature following incomplete aëration of the lungs.

The other organs showed no lesions which could in any way be interpreted as having a bearing on the disease of the liver and kidneys.

To summarize then, briefly, in two small children of the same parentage there was found a condition of hyperplasia of duct epithelium with a tendency to cyst formation especially marked in the kidneys and the liver, where there was also an increase in the number of ducts, but occurring also to a less degree in the pancreas and possibly in the intestine.

The attempts to explain the pathogenesis of the congenital cystic kidney have been so numerous and so varied that one is inclined to question whether pathologists have been dealing throughout with a single pathological process. Though different processes may be operative in the condition it seems

[21] *Festschrift der Phys.-Med. Gesellsch.*, Würzburg, 1899.

safe to assume that but a single one accounts for the group in which both kidney and liver are affected, and it is to this group that the present case belongs. Any light that may be gained from a study of it may accordingly, it would seem, be applied to the whole group.

If one omits the view that the cysts are formed in the interstitial tissue of the kidney which was early abandoned, and confines himself to those theories which recognize their formation either in the tubules or in the glomeruli, the attempted explanations of the pathogenesis of the condition fall naturally into three or possibly four groups:

(1) that the cysts are the result of obstruction and of retention of secretion;
(2) that they are of the nature of a neoplasm;
(3) that they are the result of malformation; and
(4) that the condition lies between the last two, partaking somewhat of the nature of each.

The literature of the subject has been extensively reviewed in many papers, notably those of Luzzatto,[22] Dunger,[23] von Kahlden,[24] and Busse,[25] and I shall make but brief reference to the more important opinions under the several headings of the above classification, acknowledging my indebtedness to these authors, where specific references are not given.

The great exponent of the retention theory was Virchow, and it has probably been the weight of his opinion that has gathered and held so many adherents to this view. There have been many variations in the theory, the contention being as to the nature of the obstruction. The general group of views may, however, be well summarized in the words of Henry Morris[26]: "Regarding as I do the congenital cystic kidney of the fœtus and the new born and the large polycystic kidney of the adult as essentially the same disease, both being due to dilatation, the result of obstruction, one must yet recognize different causes of obstruction

[22] Loc. cit.
[23] Loc. cit.
[24] *Ziegler's Beiträge*, 1893, iii, 291.
[25] *Virchow's Archiv*, 1904, clxxv, 442.
[26] Loc. cit.

in different cases some congenital, some inflammatory, some malformation, some traumatic, some in the kidney, some in the ureter, and some in the urethra. The affection may commence in utero or during the first months of extrauterine life, or in early childhood, or not until middle age, or advanced, or quite old age."

Virchow[27] first advocated the view that uric-acid deposits were the cause of the obstruction but in a later paper[28] advanced the theory that the lesion was the result of a fœtal papillitis or pyelonephritis which caused an increase in the interstitial tissue of the papilla. This in its contraction resulted in the atresia of the papilla and thus in the formation of retention cysts in the tubules. To this view he still adhered in 1892.[29] Frerichs attributed the cysts to retention following obstruction by casts; Bouillard and Lehmann to obstruction by uric acid and calcium salts; Klein, Rosenstein, and for a time Brigidi and Severi, to hæmorrhage from the glomerular tufts with obstruction of the outlet and dilatation of the capsule. Sabourin, Cornil, and Brault attributed the lesion to an inflammatory condition in the kidney resulting, through an interstitial new growth of tissue or cirrhosis, in a constriction of tubules and retention. The retention theory has had many other supporters who see in the cysts of the kidneys of chronic nephritis a lesion analogous to that of the congenital cystic kidney.

In 1875 Sturm[30] expressed the view that the origin of the cysts lay in the epithelium of the convoluted tubules, which at first as the result of a dilatation underwent a simple hypertrophy but later led to the putting out of bud-like processes of an adenomatous nature. By the early fatty degeneration of these constantly growing tumor-like processes, the cysts were formed. In 1876 Mihalkowicz[31] expressed the view that the condition in the kidney was analogous to the cystic disease of ovary, testicle, and breast. Chotinsky[32] could find no evidence that the

[27] *Verh. der Gesellsch. f. Geburtshülfe*, Berlin, 1847, ii, 170.
[28] *Verh. der. Phys.-Med. Gesellsch.*, Würzburg, 1855, v, 447.
[29] *Berlin. klin. Woch.*, 1892, xxix, 105.
[30] *Arch. der Heilkunde*, 1875, xvi, 193.
[31] Thesis, Paris, 1876.
[32] Thesis, Bern, 1882.

glomeruli took part in the cystic formation, but found signs, instead, of an active growth of the tubules in outgrowths from the tubules, solid cellular processes, and probably new formation of tubules. He concluded the primary factor was a hyperplasia of the tubules and of the stroma. Lejars[33] described the condition as a persistent congenital lesion, in the great majority of cases of the type of an "Epithelioma Mucoïde." In 1893 Nauwerk and Hufschmid[34] recognized buds from the tubules, intracystic papillary growths, and solid nests of epithelial cells and placed the lesion definitely in the group of adeno-cystomata. Von Kahlden[35] supported this idea, but emphasized farther the coördinate growth of the stroma analogous to that in the adeno-fibromata. He considered the lesion of cystic liver of the same nature. There are numerous other supporters of the theory of active growth of the epithelium of the tubules, among them Hommey, Hausmann, Albert, and Schmitz.

The frequent association of the congenital cystic kidney with malformation of various parts and organs has led to the idea that it too is to be regarded as a malformation or the result of an error in development. This view gains some support from the extreme complexity of the process of development of the genito-urinary system as a whole. Koster[36] was the first to describe the lesion as an error in development, but he based his theory on a faulty embryology. With the assumption that the tubules of the kidney grew from a separate "anlage" from that of the pelvis and joined it secondarily, he postulated a failure of union between the two parts and a consequent hyperplasia of the epithelium of the tubules with the cystic result. Hildebrand[37] has made use of quite a similar explanation on the basis of the modern dualistic theory of the formation of the kidney, i.e., that glomeruli and convoluted tubules arise from one anlage and secondarily join the collecting tubules which are an outgrowth from the pelvis. Hildebrand postulated a failure of union between these two

[33] Thesis, Paris, 1888.
[34] *Ziegler's Beiträge*, 1893, xii, 1.
[35] *Ziegler's Beiträge*, 1893, xiii, 291.
[36] Quoted by Dunger.
[37] *Archiv für klin. Chirurgie*, 1894, xlviii, 343.

systems of tubules, with a retention of secretion and cyst-formation in the convoluted portion. Ribbert[38] has made use of the same facts of development to explain the pathology of the condition, but has emphasized, instead of retention of secretion as the causal formation, a wild excessive proliferation of the cells of the tubules which fail to join, particularly of the ampulla of the collecting tubules and the portion of the other section, corresponding to the junctional tubule. He calls attention also to the point that in this active hyperplasia of the epithelium the condition bears some resemblance to a new growth. Meyer[39] and Busse[40] express a similar opinion. Shattock[41] attributed the lesion to the development of retention cysts from portions of the Wolffian body included within the kidney. Bard and Lemoine[42] expressed the opinion that the lesion was due not to a primary epithelial hyperplasia, but to a defective resistance of the walls of the tubules, in particular of the basement membrane, which allowed dilatation under the normal pressure of the secreted fluid they contained.

Quite a group of recent writers have taken what may be termed a middle position between the neoplastic and malformation theories. Thus Couvelaire[43] objects to the consideration of the lesion as a tumor of the cystoma or adenocystoma type, and concludes that the process which leads to the cystic transformation consists essentially in an exuberant but general proliferation of the epithelial cells of the excretory ducts, with a correlated reactive growth of the connective tissue. The specific character of the cells is lost. This hyperplasia and perversion in the evolution of the cellular elements take place while the organ is developing and is to be considered as a malformation in that sense. Borst,[44] though retaining the lesion among the cystic adenofibromata, considers the process as an error of development,

[38] *Verh. der deutsch. path. Gesellsch.*, 1899, 2te Tagung, 187.
[39] *Virchow's Archiv*, 1903, clxxiii, 209.
[40] Loc. cit.
[41] *Trans. of the Path. Soc.*, London, 1886, xxvii, 287.
[42] *Arch. de méd.*, 1890, ii, 151.
[43] *Annales de gynêc. et d'obstet.*, 1899, lii, 453.
[44] Loc. cit. and Die Lehre von der Geschwülsten, 1902, p. 596.

not in the sense of Ribbert, but in a disturbance of equilibrium between the growth of epithelium and connective tissue, the *sine qua non* for the normal development of an organ. In the cystic liver and kidney, he postulates a failure of the epithelium to develop in a normal way, as the result of a pathological agent, and to possess an increased power of growth. As a result, the connective tissue is invaded. Luzzatto [45] concludes that the pathogenesis of the cystic kidney is not uniform: that in a small number of cases—the shrunken cystic kidneys—the lesion is a result of an interstitial nephritis of fœtal origin; that in the more common type it is to be regarded as either a malformation or a tumor of congenital origin, between which he is unable to draw a sharp line. Still he is inclined to include four congenital cases of his study under the fibrocystadenomata, and a fifth under malformations, due to an excessive development of connective tissue in the organ.

Dunger [46] recognizes an inflammatory type, but also places the more common in the unsettled province between congenital neoplasms and malformations. He considers the primary factor, however, an error in development, with a secondary proliferation of the epithelial and connective tissues.

In considering these theories from the standpoint of the cases here presented, one notices first that the kidneys offer no evidence whatever in support of the obstruction and retention theory. The urinary path was open throughout from pelvis to urethral orifice. The children lived eleven and nineteen days respectively and passed urine freely. In the kidney the microscopical sections showed the orifices of the ducts of Bellini free and if anything wider than normal. In the kidneys of child "B" there was practically no increase in the interstitial tissue to cause constriction of the tubules. The tubules were in great part free from other than fluid content, and uric-acid deposits and casts, though present, were for the greater part in the least dilated tubules.

While studying these cases, the author has had opportunity

[45] Loc. cit.
[46] Loc. cit.

to compare the picture afforded by the congenital cystic kidney with that of a congenital hydronephrosis following a stricture of the ureter (Autopsy 2504), in a child which lived but a few days after delivery. The sections of the two lesions were entirely different. In the hydronephrosis there was but moderate dilatation of the ducts of the pyramids, with beginning flattening of the papilla and a beginning proliferation of the interstitial tissue near the mouths of the ducts. There was no indication of active growth of the epithelium. Further, the dilatation of the tubules consequent on the damming back of the urine was noticed not only in the medulla, but also in the cortex, in the convoluted tubules where the lumen was wide and the epithelium somewhat flattened, and even in the glomeruli where the capsules were considerably dilated. In the congenital cystic kidneys, on the other hand, the tubules of the secreting type in the cortex and the glomeruli, with very few exceptions, were entirely unaffected.

These cases then give absolutely no support to the theories which refer the lesion to obstruction and retention. There is as little evidence afforded to support the idea of any malformation or error in development of the kidneys in the sense of Hildebrand and Ribbert—that is, of a failure of the two sets of tubules to join with each other. The glomeruli and cortical tubules were well formed and apparently normal in number. The loops of Henle were possibly somewhat shorter and less well developed than in kidneys of an equal age, but this was the only possible deviation from the normal. If there were a failure of union, one would expect a dilatation of the convoluted tubules or glomeruli due to retained secretion. But in these kidneys no such dilatation was found, and, though the cystic formation was diffuse throughout the kidney, there was anatomical and physiological evidence that the urinary path from glomerulus to pelvis was free.

The sole malformation, if it may be called such, was an active proliferation of the epithelium of the collecting ducts of the medulla and of the junctional tubules in the cortex, chiefly in the inner part. Of such active proliferation, there was positive evidence in the numerous mitotic figures found in the epithelial

lining of the cysts in child "B." The absence of an increase of connective-tissue growth in the kidney of this child and its presence in that of the other seems evidence also that such sclerosis is a secondary phenomenon. In the kidney I was unable to satisfy myself that there was an excessive formation of tubules. In the liver, on the other hand, where that point is more easily determined from the architecture of the organ, the ducts were not only larger than normal but much more numerous, and in many cases penetrated the lobules almost to the central vein. There seems no question that one must consider the processes in the two organs as essentially the same, and this suggests the possibility that some of the cystic tubules in the labyrinth of the kidney cortex, which clearly showed a connection with the ducts of the medullary rays, but which much less rarely could be found to continue into tubules of the secreting type, were new-formed tubules, analogous to those ducts of the liver which penetrated the lobule. The condition in some particulars thus resembles a neoplasm, yet it is as readily considered a malformation in the sense of an abnormal power of growth of the duct epithelium. If one accepts the Cohnheim theory for the origin of certain tumors in the congenital misplacement of tissues, an error in development, there is no sharp line between the two conditions. However, it seems to the author scarcely appropriate to term the congenital cystic kidney an adenocystoma. Instead of a localized growth more or less sharply outlined from normal tissue, one finds here a diffuse and widespread influence affecting the conducting tubules generally in the kidney, liver, and pancreas, and even possibly in the intestine. The occurrence of multiple tumors in the body is not unusual, but it is to be regarded more or less as a coincidence, and in no group of tumors do we have as constant bilateral occurrence as in congenital cystic kidneys, or as frequent an association as is found between that lesion and the cystic liver. Further, in the several organs the abnormal tubules were directly connected with normal parenchyma, and in the kidney there seems physiological and anatomical evidence that in the cases here reported the cystic tubules carried on their normal function of carrying away the secretion from the parenchyma of the

organ. That this functional activity persists for any great length of time I do not maintain. It is readily seen that the increase in the size of the cysts, their encroachment on each other, and the coördinate growth of connective tissue might soon lead to obstruction of a great number of tubules and ducts. This, however, would be a secondary effect.

Primarily, then, one finds evidence of an epithelial hyperplasia in ducts which otherwise appear normal. This leads at first to a general cylindrical increase in the size of the ducts, but gradually there is a greater dilatation at points in the ducts less fixed by the architecture of the organ, and cystic tubules are the result. The expansile force must at first be the normal pressure of the secreted urine, a force which secondarily becomes much greater owing to the mutual pressure of the dilated tubules and consequent obstruction of some of them at various points. It may be objected that a primary weakness of interstitial supporting tissue has not been excluded as the stimulus to the epithelial hyperplasia. To explain why one finds the cystic formation instead of intratubular epithelial growths in case of a primary epithelial hyperplasia, one must assume that the normal urinary pressure is greater than the resistance offered by the supporting kidney tissue. However, that there is any abnormal weakness of this tissue does not necessarily follow, and in fact the specimens offer no evidence of any deficiency in the supporting membrane of the tubules. The tortuosity of certain dilated ducts in the boundary zone, where there appears to be the greatest resistance to expansion, seems an added point in the favor of a primary epithelial process. The position of the lesion in pathological classification would seem to depend on a definition of terms. If it be a neoplasm, it differs from the conditions usually included under that term. If it be a malformation, it is one only in the sense of Couvelaire and Borst—a disproportionate activity in the growth of the epithelium. The frequent occurrence of associated malformations of other parts and organs would appear to indicate that the condition bears a closer relation to malformations than to new growths in the commonly accepted interpretation of the latter term.

From the marked hereditary and family tendency shown in the disease, one is led to search for the etiological factor in the parents. Where the condition has appeared in two generations it has generally been transmitted from father to son and from mother to daughter. Where it appears in several members of one generation, several successive children of either sex may be affected or healthy children may intervene. In one case, Singer's, there was a definite regularity in the appearance of the disease, every third child of thirteen having shown cystic kidneys. The condition then in the parent which determines the lesion in the child is not always operative. What this factor is must for the present be left undetermined.

The abbreviated autopsy protocols of the two cases are as follows:

Autopsy No. 2291. April 28, 1904. Post-mortem examination on Baby F. (child "A"), aged 11 days.

The body is that of a small but well-formed colored male infant, 44 cm. in length. There is some desquamation of the epidermis from the arms and hands, and there are signs of nasal discharge. The abdominal cavity shows a slight excess of clear fluid with marked œdema of the retroperitoneal tissues. The pleural and pericardial cavities are normal.

The heart is of about normal size, and, except for marked distension of the coronary veins, is normal throughout.

The lungs are of pinkish color, deepening to a red in the posterior portion, and crepitate throughout. On section they show marked congestion of the vessels, and the cut surface is moist.

The spleen measures 4 x 2.5 x 15. cm. and is dark red in color. On section the pulp is increased in amount, obscuring the architecture of the organ; Malpighian corpuscles visible.

The liver is about normal in size with smooth regular surface. On section it has a dark purplish color, mottled with areas of light grayish translucent appearance, which are leaf-like in shape. These areas seem firmer than the liver parenchyma. There are scattered throughout the organ also, small light colored areas which are just visible. The limits of the lobules cannot easily be made out. There is an increase in the connective tissue about the larger vessels. The gall bladder and ducts appear normal.

The pancreas, stomach, and intestine show no definite lesions.

The kidneys are described on page 276.

Adrenals, testicles, and epididymes show no lesions.

The lines of ossification in the femur and tibia are sharp, clean cut, and regular

Aorta and branches are normal.

Microscopical examination. With the exception of the organs described no lesions of importance were found.

Liver. See page 278.

Pancreas. The secreting parenchyma is well formed. Many of the cells contain clear spaces and vacuoles. The ducts are prominent in the section, are dilated, are more branched, and seem comparatively more numerous than in other pancreases of this age.

Kidney. See page 277.

Anatomical diagnosis. Congenital cystic kidneys and liver; catarrhal enteritis. Acute splenic tumor.

AUTOPSY 2515. April 10, 1905. Baby F. (child "B"), aged 19 days.

The body is that of an emaciated colored female child, 42.5 cm. in length. There are no external lesions.

The serous cavities show no lesions.

The heart is of about normal size, and the cavities are filled with rather thick unclotted blood. The valves, endocardium, and myocardium are normal.

The left lung is of light pinkish color, is dry on section, and crepitates throughout. The right lung resembles the left.

The spleen is enlarged and measures 5 x 3 x 1.5 cm. On section the pulp, which is of dark brownish red color, seems increased in amount. The Malpighian corpuscles are enlarged.

The liver is small measuring 10 x 5.5 x 3 cm. The surface is smooth and glistening and the organ seems normally formed. The color is a deep brown. On section the brownish surface shows everywhere leaf-like grayish translucent areas, apparently representing enlarged portal spaces. Otherwise the architecture of the organ cannot be made out. The gall bladder contains a small amount of thick greenish bile. The ducts appear normal.

The pancreas appears normal in size and texture, with no increase in its stroma.

The stomach is distended. Its mucosa is swollen, injected, and covered with thick mucus.

The intestine shows a greenish fluid fæcal content, and considerable mucus on its epithelial surface. The mucosa is injected at intervals throughout its length, and is swollen and velvety.

Mesenteric lymph glands are enlarged, opaque whitish in color, and soft and moist to the touch.

The left kidney is slightly enlarged, and measures 5 x 2.7 x 2.5 cm.; the right measures 4.5 x 2.8 x 2.4 cm. For description see page 275.

The adrenals show no lesion. The bladder and genitalia are normal throughout.

The lines of ossification at the lower end of the femur and upper end of the tibia are sharp and clean cut, not thickened or irregular.

MICROSCOPICAL EXAMINATION—Liver. See page 277.

Pancreas. See page 278.

Duodenum. See page 278.

Kidney. See page 275.

The lesions in the other organs are not of interest.

Anatomical diagnosis. Congenital cystic kidney, liver, and pancreas; acute catarrhal gastro-enteritis; acute splenic tumor; acute mesenteric lymphadenitis.

THE RESUSCITATION OF THE CENTRAL NERVOUS SYSTEM OF MAMMALS.*

By G. N. STEWART, C. C. GUTHRIE, R. L. BURNS, AND F. H. PIKE.

From the Hull Laboratory of Physiology, the University of Chicago.)

Introduction.
Technique of the Experiments.
The General Results of Occlusion of the Cerebral Arteries.
The General Phenomena following Restoration of the Cerebral Circulation.
Summary and Conclusion.

INTRODUCTION.

The literature on anæmia of the central nervous system is extensive. A review of the work on cerebral anæmia up to 1900 is given by Hill.[1] Hayem and Barrier[2] review the work on resuscitation of the isolated brain up to the time of publication of their results. Further summaries of previous work on resuscitation in general are given by Batelli,[3] Prus,[4] and d'Halluin.[5] It does not therefore seem necessary to review the earlier studies. We shall proceed to a description of our own experiments.

A preliminary account of some of the present series of experiments has been given by Drs. Stewart and Guthrie.[6] Our ob-

* The experiments described in this paper were begun by Prof. Stewart and Dr. Guthrie, continued by Dr. Guthrie, Mr. Burns, and Mr. Pike, and concluded by Mr. Burns and Mr. Pike. The paper was written largely by Mr. Pike, with tabulations by Prof. Stewart.

[1] Hill, *Philosophical Transactions of the Royal Society*, 1900, 193, B.

[2] Hayem et Barrier, *Archives de physiologie normale et pathologique*, 1887, x, 3e serie, 1.

[3] Batelli, *Comptes rendus de l'Académie des Sciences*, 1900, cxxx, 800; *Journal de Physiologie et Pathologie générale*, 1900, ii, 443.

[4] Prus, *Wiener klinische Wochenschrift*, 1900, xiii, 451, 482; *Archives de médicine experimentale et d' anatomie pathologique*, 1901, xiii, 352.

[5] D'Halluin, *Comptes rendus de la Société de Biologie*, 1904, lvii, 66; *Résurrection du cœur ; la vie du cœur d isolé; le massage du cœur*, 1904, 118; Th. Lille. *La Presse Médicale*, 1904, xii, 345.

[6] Stewart and Guthrie, *Science*, 1905, xxi, 887.

ject has been to gain, at first hand, a knowledge of the condition of the anterior part of the cord and of the brain centers during total acute anæmia, and to determine the ultimate limit at, or below, which resuscitation is possible.

TECHNIQUE OF THE EXPERIMENTS.

Nearly all the experiments were done on cats. A few of the early experiments were done on dogs. In the earlier experiments in which blood-pressure changes, and the disappearance of reflexes were the only things desired, a tracheal cannula was inserted. In experiments in which it was desirable to keep the animal alive some hours or days after occlusion, a glass tube, narrowed at the end, was inserted through the mouth and between the vocal cords into the larynx after narcosis by inhalation. This latter method of intubation we adopted later as a routine procedure, it being less difficult and requiring less time than tracheotomy. The tube is retained in position by means of a strong cord tied about the lower jaw. Ether, the only anæsthetic used, was administered through the tube. Artificial respiration was accomplished by slipping the free end of the air tube, from a tank of compressed air, with an escape valve in the side, over the end of the glass tube.

Through an incision in the median line in the lower part of the neck, the right innominate and left subclavian arteries were secured. Careful dissection is required to avoid rupturing the pleuræ and the thoracic duct, both of which lie in the field of operation. The truncus thyreo-cervicalis may be mistaken for the vertebral artery.

The manner of origin of the cerebral arteries is of importance in relation to the production of cerebral anæmia. According to Chauveau,[7] the general form of origin of the carotids in vertebrates below man is by the bifurcation of a common trunk (tronc cephalique) arising from the arteria anonyma. (Fig. 1, a.) Quite as often, however, we have found the left carotid arising separately from the arteria anonyma, and the right carotid and right subclavian arising by the bifurcation of a common trunk. This latter form is given as the usual one for the cat by Wilder and Gage,[8] and Reighard and Jennings.[9] (Fig. 1, c.) We

[7] Chauveau, *Traité d'anatomie comparée des animaux domestiques*, 3e ed. par S. Arloing, Paris, 1879.

[8] Wilder and Gage, *Anatomical Technology*, 2d ed., New York, 1886.

[9] Reighard and Jennings, *Anatomy of the Cat*, New York, 1901.

have found, on post-mortem examination, a third form of origin, intermediate between the other two, in which both right and left carotids and the right subclavian artery arise from a common point (Fig. 1, b.) In one case, the left subclavian gave off a branch, of uncertain distribution, proximal to the origin of the vertebral.

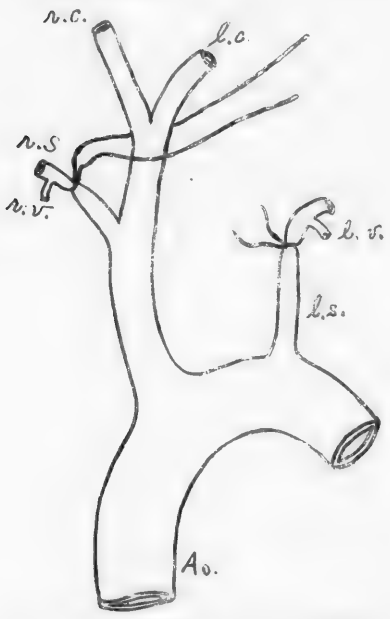

FIG. 1 (*a*). Showing the tronc cephalique of Chauveau.

Ao., aorta; r.s., right subclavian; r.v., right vertebral; r.c., right carotid; l.c., left carotid; l.s., left subclavian; l.v., left vertebral.

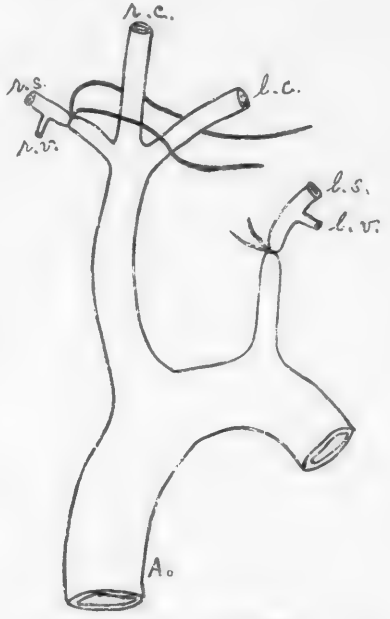

FIG. 1 (*b*). The intermediate type of branching.

Ao., aorta; r.s., right subclavian; r.v., right vertebral; r.c., right carotid; l.c., left carotid; l.s., left subclavian; l.v., left vertebral.

Acute temporary cerebral anæmia was produced by passing ligatures around the innominate, and the left subclavian proximal to the origin of the vertebral artery. Traction on the ligatures produced occlusion of the arteries. In case of anomalous origin of the arteries, complete occlusion is assured only when both carotids are clamped separately. In a few experiments the aorta was clamped proximal to the origin of the arteria anonyma.

To keep the animals alive during, and for some time after, the period of occlusion, artificial respiration was employed. The apparatus used for this purpose has proved to be so valuable that we describe it in some detail. An ordinary musician's

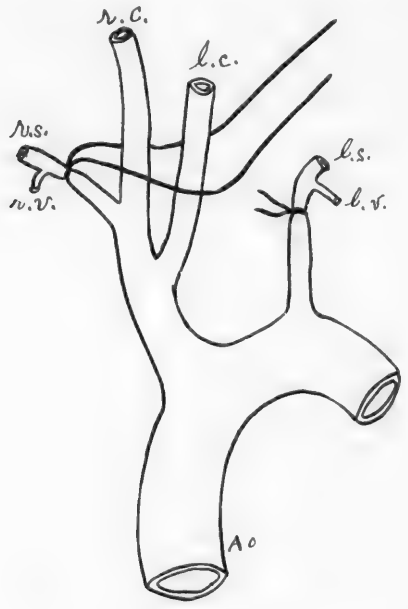

FIG. 1 (c).
Ao., aorta; r.s., right subclavian; r.v., right vertebral; r.c., right carotid; l.c., left carotid; l.s., left subclavian; l.v., left vertebral.

metronome was fitted with mercury cups and platinum-wire contacts so as to make and break an electric circuit at each complete vibration. The current from one or two batteries was conducted through the metronome and an ordinary telegraph relay and a time marker in circuit with it. This first relay made and broke the circuit through a second relay operated by the electric-light current. A wire soldered to the bar of the second relay operated a valve in the compressed air tube, the distal end of which was connected with the glass tube in the cat's throat.

Any desired rate of breathing could be obtained by adjusting the metronome.

The blood pressure was always taken from the left carotid artery unless otherwise stated. Sodium-citrate solution was used to prevent coagulation of the blood in the arterial cannula.

When it was desired to keep the animal alive for some time the experiment was done under antiseptic conditions. The neck wound was closed with silk ligatures, sealed with collodion, and bandaged.

Respiratory tracings were made by using the usual tambour connected with the throat tube.

THE GENERAL RESULTS OF OCCLUSION OF THE CEREBRAL ARTERIES.

Tests of Occlusion.—The phenomena of complete occlusion are characteristic and constant. The nose and mucosa of the mouth become white as in death. Respiration ceases, the reflexes disappear, and the pupils dilate completely. The heart is but little affected. Intravenous injection of indigo carmine showed, on post-mortem examination of animals allowed to die without restoration of the cerebral circulation, that anæmia of the brain and medulla oblongata was complete in animals showing these symptoms; that is to say, no trace of the pigment was ever found above the level of the calamus scriptorius. Usually the pigment extended no higher than the third or fourth cervical segment.

Occlusion of the cerebral arteries does not always seem to produce total anæmia, owing, it is to be presumed, to a greater collateral circulation through the spinal arteries. In any event, in a certain number of cases the typical symptoms which we associate with total anæmia are not observed. The persistence of respiration for some time, up to eight minutes, the tardy disappearance of reflexes, and the oozing of a little blood from a nick in the nasal septum distinguish these cases from the first and much the larger group. The disappearance of the reflexes will be considered in detail later in the paper.

Blood-pressure Changes.—When the cartoid artery of the animal

operated on is connected with a mercury manometer, one of the most striking effects of occlusion of the cerebral arteries is the rapid and great rise in blood pressure. There is at first a rise above the general pressure before occlusion, then a fall, succeeded by a second rise and a second rapid fall to the level which is maintained, with only a very gradual fall, throughout the period of occlusion. The numerical values together with the time relations of each are given in Table I.

TABLE I.

Number of experiment	Pressure before occlusion.	First rise to	First rise in	First fall to	First fall in	Second rise to	Second rise in	Second fall to	Second fall in
1	82 mm. Hg.	102 mm.	19 sec.	62 mm.	32 sec.	114 mm.	158 sec.	40 mm.	270 sec.
2	58 " "	112 "	32 "	46 "	23 "	140 "	115 "	54 "	115 "
3	126 " "	156 "	30 "	148 "	60 "	220 "	78 "	100 "	330 "
4	144 " "	174 "	9 "	104 "	30 "	190 "	87 "	64 "	95 "
5	85 " "	103 "	15 "	70 "	13 "	110 "	45 "	20 "	85 "
Mean	99	120	21	98	32	155	97	56	159

Plotting the means of these results, using time as abscissas and pressure as ordinates, we obtain the composite blood-pressure curve shown in Fig. 2.

It will be noticed, on examination of the curve, that (1) the first rise is relatively rapid; (2) the first fall is slightly slower, and sometimes reaches a point below the pressure before occlusion; (3) a second, slower, and usually greater, rise follows, and finally there follows (4) the second fall to a point below the pressure before occlusion, sometimes almost to zero.

The character of the curve is altered when the vagi are divided, the first rise occurring as usual but without the first fall and the second rise.

Section of the cord in the cervical region, as soon after occlusion as possible, causes a prompt and profound fall in pressure.

When both vagi and cord are divided before occlusion a feeble rise of about one centimeter occurs.

To determine the mechanism by which this rise in blood pressure is brought about, we have made a number of experiments. When the abdominal wall is opened, the mesentery exposed and afterward protected from cold, the splanchnic vessels may be observed to contract somewhat, following occlusion. The fall in blood pressure resulting from section of the cord shortly

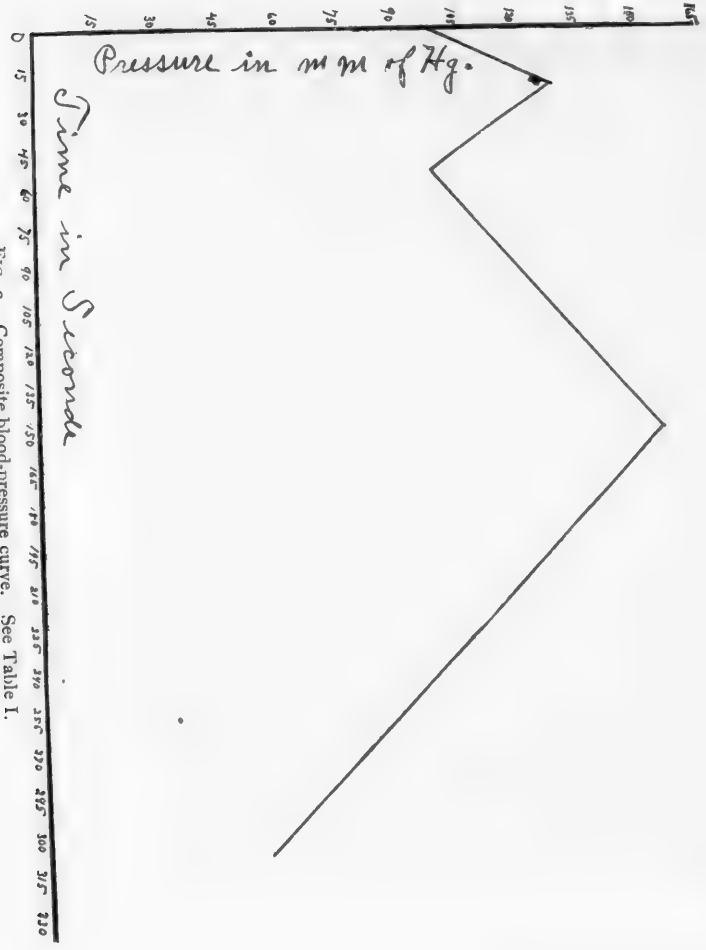

FIG. 2. Composite blood-pressure curve. See Table I.

after occlusion affords further evidence of vaso-constrictor agency in causing the usual rise; and the relatively small rise in blood pressure following occlusion after division of the cord is conclusive proof of the action of the medullary center.

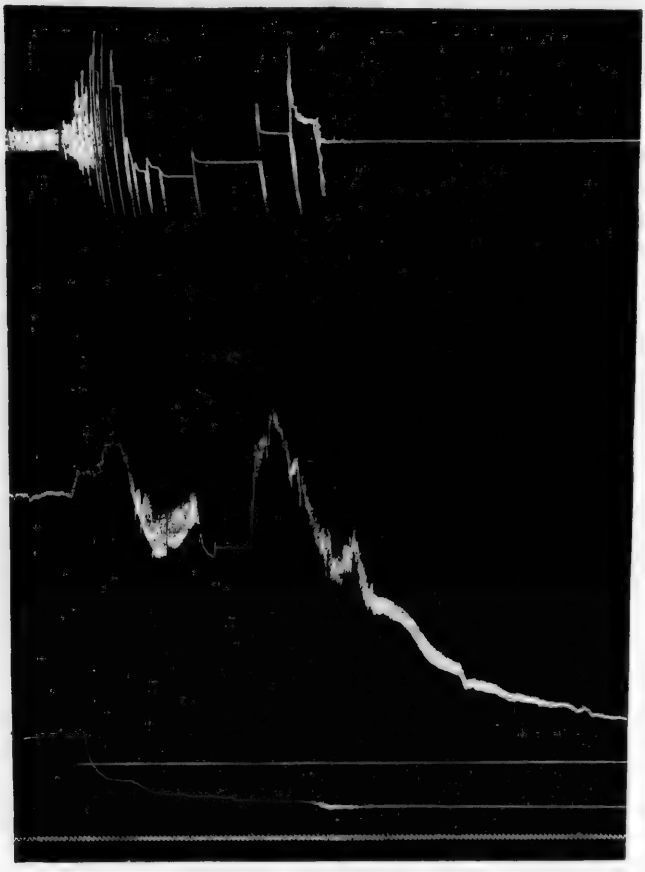

Fig. 3.—Time tracing in seconds. Cannulæ in central and peripheral ends of left carotid. Base line for peripheral (cephalic) pressure first above time tracing; base line for central (cardiac) pressure second above time tracing. Curve of cephalic pressure crosses base line for pressure from central end. Respiration at top, above blood pressure. No artificial respiration. (Expt. 66.)

The cause of the first fall and the second rise we believe to

be twofold. (1) When a typical blood-pressure curve (vagi intact) is examined, an increased oscillation of the manometer is observed to begin near the end of the first fall and to continue until near the crest of the second rise (Fig. 3). Pulse counts taken at intervals during occlusion, and a blood-pressure tracing on a rapidly moving drum show that a period of cardiac slowing intervenes here. This inhibition begins at the crest of the first rise, becomes most prominent about the end of the first fall, and then gradually weakens until it disappears entirely at the crest of the second rise.

Bastgen[10], by the injection of oil into the peripheral end of the carotids, and S. Mayer,[11] by the ligation of the cerebral arteries, produced a high blood pressure with slow pulse. Mayer's curves show a distinct vagus inhibition, but without such a marked fall as in our experiments, although he describes a first rise, a fall, a second rise, and a second fall. He ascribes the rise in pressure to (a) a diminished outflow from the heart and (b) an increased inflow from the veins. Bastgen believes that the fall in pressure is due to vagus inhibition, —a suggestion which we adopt as a provisional explanation of the first fall.

(2) Mayer[12] showed that, in Cooper's experiments, the respiratory and vaso-constrictor centers were more tenacious of life than the other cerebral centers. Schiff[13] showed that the accelerator fibers in the vagus resist degenerative changes longer than the inhibitory fibers. Arloing,[14] who repeated Schiff's work, says there are four kinds of fibers in the vagus, one kind being the accelerator fibers, and confirms Schiff's statement as to their greater resistance to degeneration. Hering[15] and more recently Danilewsky[16] have shown that the accelerators retain their vitality longer after the death of the animal (rabbits), than the inhibitory fibers. It is conceivable that the accelerators are more resistant to anæmia than the inhibitory fibers. The second rise in pressure

[10] Bastgen, Dissertation, Würzburg, 1879.

[11] Mayer, *Sitzungsber. der Kaiser. Akademie der Wissenschaften zu Wien*, 1879, lxxix, 3, Abthl., 87.

[12] Mayer, *Medicinisches Centralblatt*, 1878, xvi, 579

[13] Schiff, *Archiv für die gesammte Physiologie*, 1878, xviii, 172.

[14] Arloing, *Archives de physiologie normale et pathologique*, 1896, viii, 5e serie, 75.

[15] Hering, *Archiv für die gesammte Physiologie*, 1903, xcix, 245.

[16] Danilewsky, *Rousski Vratch* (*Le Médicin russe*), 1904, iii, 641.

is caused by the action of the vaso-constrictor center, and may also be due in part to the action of the accelerators in the vagus. The condition of the vagus center will be considered further in the discussion of the pulse rate.

When the animal has been under anæsthesia for a prolonged period, or when the heart and the respiratory centers have been resuscitated after failure from any cause, or when, for any reason, the vaso-constrictor center in the medulla is in an unstable condition and the Traube-Hering curves appear in the blood-pressure tracing, the first rise of pressure following occlusion and the first fall may be slight or inconsequential, but the slowing of the heart, and the second rise occur as usual. At times, occlusion is followed by the usual first rise, but the first fall in blood pressure is slight, although vagus inhibition may be marked.

After division of the vagi, the first rise in pressure occurs as usual after occlusion, but the period of cardiac slowing and the first fall in pressure are generally absent. We have observed, as did Mayer,[17] that occasional evidences of inhibition are present after section of both vagi. The pathway of such inhibitory influences is unknown to us. It is possible that the inhibition arises from asphyxia from imperfect adjustment of the apparatus. More rarely, the blood-pressure curve following occlusion after section of the vagi approaches the usual form with the vagi intact.

When the cervical cord and the vagi are severed before occlusion, a marked rise of pressure occurs at the instant of division of the cord. A profound fall in pressure follows, then a period of oscillation succeeded by a period of stationary pressure. Occlusion at this time was followed immediately by a slight rise in pressure, approximately 15 mm. of mercury, which persisted throughout the period of cerebral anæmia and fell at the time of release of the cerebral vessels. There was no evidence of inhibition during occlusion. Asphyxiation of the same animal a few minutes later was followed by a curve resembling the usual curve of occlusion, but beginning much more tardily and not reaching as great a height. We are inclined to the opinion that the rise

[17] Mayer, loc. cit.

in pressure following occlusion in this experiment was purely a mechanical effect, i.e., due to confining a given volume of blood in a smaller system of vessels.

Division of the ansa subclavia (Vieusseni), together with all communicating branches from the cervical sympathetic to the vagus below the ganglion nodosum, does not greatly modify the form of the blood-pressure curve following occlusion. In one such experiment, the period of cardiac slowing appeared to be absent, but we have not been able to confirm this result.

In the light of our own results and those of previous observers, we conclude that the vaso-constrictor center in the medulla follows in the wake of the other cephalic centers and loses its power of functioning, but the blood pressure is maintained at a height of 40 to 60 mm. of mercury until the end of occlusion. We decapitated one animal after occlusion. A fall in pressure followed,but, on the intravenous injection of 0.9 per cent. sodium-chloride solution to make good the loss of fluid by hæmorrhage, the pressure rose to 70 mm. of mercury. We conclude that blood pressure is maintained by accessory vaso-motor centers after the medullary center loses its power of functioning.

The Pulse Rate.—The period of vagus inhibition has been mentioned in connection with the blood-pressure changes during occlusion. Reference to the tables will show that, compared with the rate before occlusion, there may be an acceleration or a diminution immediately after occulsion. From the beginning of the period of occlusion on to its close there is a well-defined pulse cycle. The rate immediately after occlusion may be increased for twenty to sixty seconds but this is not a constant occurrence. The period of vagus inhibition follows and with it are associated the secondary respiratory gasps, after which the inhibition gradually weakens and ceases entirely, simultaneously with the gasps, both inhibitory and respiratory centers succumbing to the anæmia at approximately the same time. (Experiment 3, Table III.)[18]. The pulse rate then increases to a maximum after which, if the period of occlusion be sufficiently prolonged, it becomes slower until the moment of restoration of the

[18] Tables II and III are given at the end of the paper.

left ansa subclavia, as will be pointed out later. Ten seconds to a minute may elapse after occlusion before the pupil begins to dilate. The movement is rapid, four or five seconds being sufficient time to allow of maximal dilation. Occasionally the pupils dilate as usual, contract as the secondary respiratory gasps begin, and again dilate to the maximum before the cessation of these gasps. In no case have we observed the contracted state of the pupils persisting longer than the respiration, a point on which we differ from Cyon.[20] We are unable to see the reason for the persistence of the reflexes for such long periods as he gives, and we suggest that cerebral anæmia in his experiments may have been less complete than our own.

Soon after the dilation of the pupils, the cornea becomes lax, then sunken and furrowed, indicating a reduced intra-ocular pressure due, possibly, to a number of factors, e. g.: (1) a decrease in blood pressure in the vessels in and around the eye; (2) the escape of fluid from the anterior chamber of the eye; (3) loss of tone of contractile tissues of the eye and (4) loss of fluid from both chambers of the eye by diffusion and filtration into the contiguous anæmic areas.

The time of the disappearance of the various reflexes, and of their return after the restoration of the cerebral circulation is given in Table II.

Placing the hand on the chest causes a fall in blood pressure, with a rise when the hand is removed. Striking the abdomen causes a fall in pressure. Pinching the hind-feet with forceps causes a rise in pressure. Similar pinching of the fore-feet gives no result. Stimulation of the central end of the left cut vagus, the right being intact, twenty minutes after the beginning of the occlusion caused a rise in blood pressure. Cutting the right vagus of the same animal two minutes later also caused a rise in blood pressure Stimulation of the central ends of the vagi, both divided, thirty-four minutes after the beginning of anæmia caused no change in the blood pressure. A stronger current applied to the tissues of the neck caused a rise.

Muscular Movements.—There is usually a sudden stiffening of

[20] Cyon, *Comptes rendus de la Soc. de Biol.*, 1900, lii, 372.

all the muscles, following occlusion, with violent movements of the fore- and hind-limbs and tail. Micturtion and defecation may occur. Then the fore-limb movements cease, shortly afterward the hind-limbs relax, and the whole animal lies limp and quiet until after the restoration of the cerebral circulation. The intravenous injection of strychnine sulphate during the period of occlusion is followed by the usual spasms of the abdominal and hind-limb muscles, but no effect, except a possible slight exaggeration of the reflexes is observed in the fore-limbs, and only when the strychnine is injected immediately after occlusion and before the period of high blood pressure. The anterior part of the cat may be completely relaxed and inert while the posterior half may show violent strychnine spasms as in the normal cat. The blood pressure rises after the injection of strychnine. The early exaggeration of the anterior reflexes again suggests the possibility of an increased flow of blood through the anastomotic channels during the period of high blood pressure, but the subsequent complete relaxation of all anterior muscles, even when twice the minimum fatal dose of strychnine is injected, indicates that the anæmia of the anterior part of the cord is very complete.

The picture presented at the close of a typical occlusion is an animal with widely dilated pupils, lax and sunken cornea, motionless eyelids, no tear secretion, no saliva, bloodless mucosa of the nose and mouth, relaxed muscles, no voluntary respiratory movements, low blood pressure, with a heart becoming slow and weak, perhaps stopping if the occlusion be long continued. The whole sequence of events resembles in a general way those found in death by hæmorrhage, as described by Hayem.[21]

THE GENERAL PHENOMENA FOLLOWING RESTORATION OF THE CEREBRAL CIRCULATION.

Blood-Pressure Changes.—The empty cerebral arteries fill rapidly after removal of the ligatures. The mucosa of the nose and mouth lose their pallor and the blood flows freely from a nick in the nasal septum. The manometer may show a fall,

[21] Hayem, *Archives de physiologie normale et pathologique*, 1888, i, 5e serie, 103.

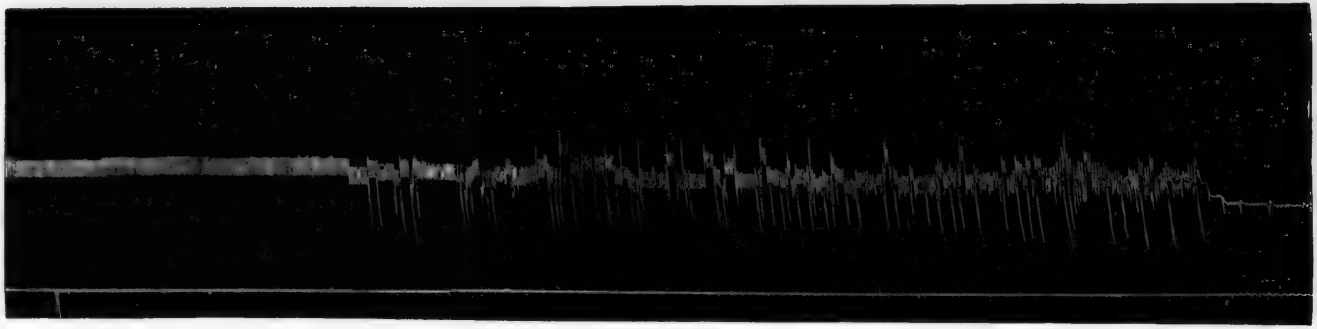

Fig. 5.—Return of the respiration. The cerebral circulation was restored at signal. Note that the first gasp, as indicated by the greater excursion of the writing point of the tambour, was a strong one. Shorter excursions of lever at right of tracing mark the beginning of natural respiration after cessation of artificial respiration. (Expt. 77.)

sometimes very marked. The pressure may even fall to zero, the heart stop, and death result. Examination of the heart, by opening the thorax immediately, may show a dilated right heart with the left ventricle in spasm.

In most cases, a slow rise in pressure succeeds the fall, continuing until the pressure before occlusion is approached or reached. Traube-Hering curves and other irregularities appear in the blood-pressure tracing. Gradually the center apparently becomes more stable in function and the irregularities disappear. Stimulation of the sciatic nerve is followed by the usual rise in pressure at this period, although it may not cause a rise in those cases where the pressure is very low and which terminate fatally.

Pulse Rate.—The rate before release of the cerebral arteries may increase after the restoration of the cerebral circulation, to diminish as the time of the first respiratory gasp approaches (Experiment 10, Table III), again increase as respiration becomes established, perhaps become very rapid, and finally, after some hours or days, return to a normal rate. The tables show pulse counts beginning at the time of release of the cerebral arteries, and continuing for five days thereafter. After prolonged periods of anæmia, the heart may gradually stop.

Respiration.—The disappearance and the return of the respiration are more definitely marked and constant phenomena than the disappearance and return of the eye reflexes.

The animal lies motionless after occlusion, with the blood pressure gradually rising. The first sign of returning vitality in the respiratory center is a strong gasp (Fig. 5) followed by another and another, the rate being about four a minute at the outset, and gradually increasing until spontaneous respiration is established. The respiratory rate is, at first, usually much slower than that of a normal cat, and hours or even days may elapse before the normal rhythm is reëstablished. After prolonged periods of anæmia, e.g., eighty-one minutes, the respiratory gasps may not occur after the release of the cerebral arteries. An occlusion of twenty-eight minutes was followed by a first gasp forty minutes after release. The gasps continued

fifteen minutes, six to eight per minute, and then ceased. Artificial respiration was continued, but dilation of the pupils and, later, failure of the heart followed. Apnœa is frequently met with following long occlusions. Respiratory gasps occur, become almost frequent enough for spontaneous respiration, and suddenly cease. Interruption of artificial respiration at such a time is followed by dilation of the pupils and collapse. If the blood is properly oxygenated, respiratory movements may begin again in twenty to sixty minutes. Cessation of artificial respiration after fifteen minutes of apnœa was followed by fifty seconds of quiet, then a spasm of the fore-legs and chest muscles, a gasp, relaxation of the spasm, and slow spontaneous respiration. In one experiment, apnœa continued for two hours.

It is worthy of note, that, in one experiment in which it was necessary to open the chest walls and massage the heart directly, ten minutes after it had ceased to beat, deep but ineffectual spontaneous respiratory gasps occurred as the blood pressure rose, each of which was followed by a sudden fall of pressure. It appears that, in this condition of the animal, a certain pressure in the respiratory center is necessary, as Hill states, to provoke respiration.

The connection between the vagus and the respiratory centers after occlusion has been noted. Pulse counts indicate a similar connection after restoration of the cerebral circulation, a distinct cardiac slowing, as has been noted, often occurring at the time of the first respiratory gasp. A very early sign of returning function occurring before respiration or contraction of the pupil is the fibrillating movements of the tongue, closely resembling fibrillation of the heart. It is observed after occlusions of ten minutes or more, and continues until the other reflexes are well established. A closely related phenomenon is the twitching of the skin of various parts of the body, but especially of the shoulders and thighs. It generally appears a little later than the fibrillating movements of the tongue, and lasts about as long.

Reflexes.—The light, lid, and corneal reflexes are inconstant. The lid and corneal reflexes may return relatively soon after release of the cerebral arteries, while the light reflex may not be

capable of demonstration until two or three days later. None of them returns after long periods of occlusion, e.g., sixty minutes. The leg reflexes are considered later. The pupillo-dilator action presents some peculiarities. We have never made an experiment in which the pupils remained contracted after failure of respiration. In cases of paralysis of the respiratory center during anæsthesia, the pupil first dilated to the maximum, the animal perhaps made two or three expulsive gasps after which the respiration failed. Respiration was always reëstablished before the pupil contracted much, but the pupil sometimes began to contract before respiration was established. After release of the cerebral arteries gasping respiratory movements appear before complete contraction of the pupil, but the pupil may be narrowly contracted before respiration is fully established, and usually remains contracted in apnœa of brief duration. It is difficult to differentiate in this condition between the action of the cerebral center, and the action of the cervical sympathetic which, as Tuckett [22] has shown, will withstand vastly longer periods of anæmia than the cerebral centers. Two and three days after occlusion may find the pupils widely dilated, while respiration proceeds without difficulty.

Intra-ocular pressure is reëstablished before, coincidently with, or more rarely after, the contraction of the pupil.

The picture presented at this stage is one in which the natural pink has replaced the deathly pallor of anæmia, the tongue is fibrillating actively, the skin twitching, pupils contracted, and respiration spontaneous but slow. The corneal, lid, and light reflexes may be present. If the period of anæmia is prolonged, the cornea may be lax and dry. The tears gather in the outer corners of the eyes and the eyelid trembles preparatory to closing completely and moistening the dull, dry cornea with the secretion which is in waiting. The cat may appear as if asleep with its eyes open, but the picture is soon to change.

The Onset of the Convulsions.—Convulsions, varying in severity from occasional twitchings of the limbs to extreme opsisthotonus and violent struggles, of tonic or clonic type, sometimes begin

[22] Tuckett, *Journal of Physiology*, 1905, xxxiii, 77.

before the full return of the reflexes. Occasionally the cat may lie quiet for one to three hours before the convulsions begin. The head may be drawn back in extreme opisthotonus, or may be bent downward with the back convex. There may be tonic spasm of the fore-limbs and clonic spasms of the hind-limbs. Again, there may be violent clonic spasms in which the animal flings itself about from one side of the room to the other. The cat may lie quietly on its side, the head in moderate opisthotonus, the fore-legs outstretched in tonic extensor spasms with alternate protrusion and retraction of the claws. If the head is gently pushed forward until it is in line with the long axis of the body, the flexor muscles then go into spasm so that the tip of the nose and the hind-feet are approximated. Soon, however, the extensor muscles overcome the flexors, and opisthotonus returns. The convulsions may follow in such rapid succession as to make it difficult to distinguish any interval between successive seizures. At other times intervals of five to twenty minutes elapse between any two convulsions. The pupils dilate widely during the spasms. Respiration may be impeded during the spasms, and rapid during the intervals. Attempts at vocalization are confined, as a rule, to the intervals between convulsions. Stroking the fur, clapping the hands together near the animal's ears, walking across the floor, passing a thermometer bulb into the rectum, and even blowing gently across the fur may throw the animal into spasms. The period of frequent and violent spasms lasts two or three hours to as many days.

Micturition and defecation occur as usual during this period. In all cases in which urine voided after occlusion has been examined, it has contained a substance which, on boiling, strongly reduces Fehling's solution. Polariscopic examination shows it to be dextro-rotatory. In one experiment, three samples of urine voided two hours and twenty minutes, three hours and thirty minutes, and twenty hours respectively, after the beginning of occlusion showed the highest percentage of reducing substance in the second sample. A very slight fermentation occurs with yeast but nothing at all proportional to the amount

of reducing substance present. The nature and cause of this condition are unknown to us.

The temperature is often subnormal, and may not rise to normal for several hours, or even days. It has appeared to us at times that a high temperature may increase the severity of the spasms, while a reduction of the temperature by putting the cat in the ice-box may reduce their severity, but of this we are not sure. The presence of a cloth covering in which the animal can entangle itself increases the severity of the spasms, as the struggles continue until the covering is thrown off. The most successful treatment consisted in keeping the animal as quiet as possible, covering it when practicable to do so without increasing the spasms, but relying mainly upon perfect quiet and freedom from jar to eliminate the spasms. Ether and chloral will stop the spasms, but may stop respiration and heart-beat also. Seizing the feet and stretching the limbs subdue the spasms during the several seconds that the limbs are held.

Severe convulsions may terminate in death twelve to thirty hours after their onset. The direct cause of death is unknown. The condition of the brain and anterior part of the cord at the end of occlusion is such as to allow of no reflexes. Fifty-two minutes after release, following an occlusion of fourteen minutes, there was no response of the anterior part of the cord to the intravenous injection of strychnine. There was no response twenty minutes after release following an occlusion of seven minutes. Strychnine at this time appeared to retard rather than accelerate the recovery of function by the anterior part of the cord. Severe strychnine spasms of the posterior half of the body followed the subcutaneous injection of strychnine, after the return of respiration and eye reflexes, thirty-three minutes after release from an eight-minute occlusion, but the anterior half of the body was relaxed. Seven hours after release from an occlusion of eight minutes, the anterior part of the cord had regained its irritability to strychnine. General spasms appeared, stronger in the hind-limbs than in the fore. On increasing the strychnine, the fore-limbs relaxed before death

while the hind-limbs were still in spasm. Another case presents some unusual features which we give in detail.

Experiment 102. Seven and one-half minutes of anæmia in the morning, with recovery, was followed by 21 minutes of anæmia in the afternoon. All reflexes returned after the second period of anæmia. Respiratory gasps began 16 minutes after release, continued 47 minutes, when a period of apnœa supervened. Artificial respiration was discontinued after 15 minutes of apnœa. The cat lay quiet 50 seconds; spasm of thorax muscles and fore-limbs, then a gasp and relaxation of spasm, and slow, spontaneous respiration followed. Left half of cord hemisected 95 minutes after release. Cat was allowed to remain undisturbed until 5 hours after occlusion. At 8 P. M., cat lay on its side, moderate opisthotonus, strong tonic spasm of fore-legs, hind-legs relaxed. Moving left hind-leg caused convulsive twitchings of both hind-legs, and mild tonic extensor spasm of right hind-leg; pupils normal; left nictitating membrane prominent. Strychnine sulphate (0.6 mg.) subcutaneously. Prick of the needle increased opisthotonus; reflexes increased, general spasms appeared, became extremely violent, and resulted in failure of respiration one hour after injection of strychnine. Fore-legs relaxed 9 minutes before respiratory failure, while spasm of the hind-legs continued up to that time. Heart stopped, all muscles relaxed, whole animal limp. Intubation and artificial respiration. Thorax opened, direct massage of heart. Heart beating regularly at 9 20, 13 minutes after its failure. At 10.40 heart was beating regularly; muscle tonus returned so much that hind-legs and tail moved spontaneously. No reflexes were obtainable anterior to posterior margin of the shoulder. Artificial respiration stopped. Auricular beats of heart observed 18 minutes afterward; no later observations were made.

The first reflex response to pulling or pinching in the front legs occurs in the leg of the same side. Later, striking one fore-leg causes reflex movements of both.

Experiment 28. Occlusion of 10 minutes, 23½ minutes after release, reflex contraction of fore-leg occurs, but only on side struck. Twelve minutes later there is crossing of reflexes, and both fore-legs contract when one is struck.

Experiment 29. Occlusion of 20 minutes; 32 minutes after release, reflex contraction of fore-leg struck, and slight contraction of opposite fore-leg. In 4 minutes more, crossing of reflexes is distinct, and both fore-legs contract when one is struck.

Experiment 32. Occlusion of 50 minutes (aorta clamped); 10 minutes after release, twitching movements of moustache. In 3 minutes, twitching movements of throat muscles over larynx where exposed in neck wound. In 33½ minutes, twitching of muscles over upper ribs in wound. In 55½ minutes twitching of skin of neck. In 67½ minutes respiratory gasps begin, involving muscles in front of neck between the shoulders, gradually spreading from below upward in the neck, including the shoulders; gasps about three per minute. 73 minutes, strong twitching of right shoulder. 77 minutes, slight spasms of shoulders and fore-limbs. Marked reflex on striking fore-limb with strong

crossed reflex of the other. 83 minutes, strong general spasm of anterior end of body. Then a strong gasp, the lower jaw coming down, breast and shoulders coming up.

It is possible that the lower portion of the cervical cord suffers less injury than the upper portion, the medulla, and the brain.

Experiment 18. Anæmia 20 minutes; 110 minutes after release, stimulation of cortex caused jaw movements of opposite side. No other movements except perhaps slight movements of opposite fore-limb. The opening of the cranial cavity was accompanied by some hæmorrhage, but as soon as this was done, deep, regular, spontaneous respiratory movements began, involving head, neck, and thorax down to the diaphragm. It was not observed whether the diaphragm participated in these movements or not. These movements were much more effective for respiration than previous respiratory movements. It is impossible to say whether trephining or hæmorrhage, by reducing intra-cranial pressure, removed the previous cause of the inhibition of the respiratory centers.

Transection of the Cord.—Division of the cord in the upper dorsal region (III to VI dorsal vertebræ), if done before fairly complete recovery of the cerebral centers, is followed by collapse, dilation of the pupils, cessation of respiration, cardiac failure, and death. The integrity of the spinal centers is necessary for the resuscitation of the cerebral centers. Intravenous injection of warm 0.9 per cent. salt solution has proved useless in averting or remedying such conditions.

Division of the cord after the spasms have become well established is followed by transient shock, recovery, and cessation of the spasms in the hind-limbs. Hemisection of the cord is followed by cessation of spasms in the hind-leg of the same side.

Experiment 50. Occlusion of 15 minutes partial, 40 minutes total, anæmia of brain. In 84½ minutes from release, strong clawing movements of hind-legs, with contraction of muscles of abdomen and thorax, up to neck, during spasms of clawing. Division of spinal cord just below last rib three hours after release. Spontaneous clawing movements of hind-limbs disappear, and do not return. Fore-limbs contract much better on pinching than before division of cord, but reflexes of hind-limbs are much less marked.

Experiments 42 and 43. Occlusion of 60 minutes. Tried to subdue spasms by ether. Death from ether 16 hours after release. Urine collected and kept in an ice-box until next day, and then injected into a half-grown cat. Needle inserted over the liver. Seven hours after, convulsions appeared in second cat; pupils dilated, hair on back and tail, erect. Spasms pass off in 30 minutes. More spasms on second day after injection. Dead on third day. We have neither carefully controlled nor have we confirmed this experiment.

Krainsky[23] has suggested that the presence of certain waste products, e.g., ammonium carbonate, in the blood may cause spasms. Donath[24] has found cholin in the blood of epileptics. More recently, Allen,[25] using a new method of testing for cholin, has failed to find it in certain nervous conditions as reported by other investigators. We have made no chemical analyses of blood or urine beyond those above mentioned. The spasms produced in the kitten by injection of urine from a cat in convulsions might be held to indicate the presence of some circulating toxin, but we do not know that the kitten had not previously suffered from spasms due to another cause. The experiments on section of the spinal cord show that whatever toxins there may be in the blood have no effect on the part of the cord not previously subjected to anæmia.

Other Structures Affected by Anæmia.—The lack of salivary and lachrymal secretion during occlusion, and the reappearance of tears have been noted above.

Experiment 32. Occlusion, 50 minutes. No return of lid or light reflexes. First gasp 67 minutes after release. Stimulation of chorda tympani 3 hours 8 minutes after release gave some secretion.

Experiment 21. Occlusion of 6½ minutes. Heart stopped 11 minutes, and again 8 minutes. Stimulation of chorda tympani 2 hours and 17 minutes after starting heart gave good flow of saliva.

In Experiment 32, 63 minutes after starting heart, stimulation of right vago-sympathetic nerve gave dilation of pupil, but only to one-half maximum size; pupil quickly contracted. Two hours and 36 minutes after starting the heart, atropine failed to dilate the pupil, even after acting a long time.

Experiment 22. Occlusion, 9⅝ minutes. Atropine dropped in left eye 2½ hours after release gave normal effect.

The chorda tympani, on stimulation, usually gives a secretion of saliva. The salivary flow returns after release of the arteries, and is often very copious. Atropine may fail to give any result. Stimulation of the vago-sympathetic may cause dilation of the pupil, but usually not to more than one-half of maximum size. The vago-sympathetic in a pup retained its power of producing dilation of pupil two hours and eleven minutes after ligation

[23] Krainsky, *Allgemeine Zeitschrift für Psychiatrie*, 1897, liv, 697.

[24] Donath, *Zeitschrift für physiologische Chemie*, 1903, xxxix, 526; *Journal of Physiology*, 1905, xxxiii, 211.

[25] Allen, *Journal of Physiology*, 1904, xxxi, 54

of the aorta. The flow of lymph from a severed thoracic duct continued throughout the occlusion.

The Deportment of the Animal in the Post-Convulsive Period.—Strictly speaking, there is no post-convulsive period except in those animals which recover completely. For convenience of description, we may speak of the period after cessation of the frequent and violent spasms, as the post-convulsive period. It is characterized by infrequency or total absence of convulsions. There are three phases: (a) complete recovery, (b) partial recovery, and (c) death.

(*a*) *Complete recovery.* Experiment 74. Very large male cat. Ether. Intubation. Occlusion of 8 minutes. Pupils dilate completely in 20 seconds, with lax cornea. Respiration ceases in 70 seconds. First respiratory gasps after release of cerebral arteries in 8 minutes. Pupils contracted in 14 minutes. Natural respiration in 18 minutes. Unusually severe spasms began 29 minutes after release, and lasted 1¼ hours. Cat was then able to crawl about, but unable to stand because of paralysis of fore-limbs. Paralysis and wrist-drop entirely gone the next morning (20 hours after release). A peculiar drooping attitude was maintained for 2 days. Afterward the cat appeared entirely normal in every way. When killed by chloroform narcosis 3 months later, lungs showed nodular thickenings and some inflammation. No gross changes in brain.

Complete recovery has resulted after occlusions of five, six, eight, nine and five-sixths, and sixteen and a half minutes.

(*b*) *Partial recovery.* The most interesting case of all comes in this group. Experiment 70. Adult female cat in advanced pregnancy. Ether. Intubation. Occlusion of 10 minutes. Pupils dilated in 15 seconds. Last respiration in 30 seconds. First respiratory gasps 11 minutes after release, spontaneous respiration in 20 minutes. Pupils contracted in 24 minutes. Corneal reflex present in 90 minutes. Convulsions began one hour after release of vessels. Extreme opisthotonus and violent clonic spasms never appeared, but convulsive movements of the limbs were present, without any intermissions, for 3½ days. She lay on her side all the time. Violent spasms resulted whenever the fur was stroked. Walking across the floor caused convulsive starts. The click of the stop watch near her ear, blowing against the fur, or any other slight disturbance caused a convulsive start. The pupils were wide, with light reflex appearing the second day.

On the morning of the fourth day, she was found in a half-sitting posture, head drawn back, and fore-paws outstretched. On the morning of the sixth day, she was able to stand and walk clumsily. Paralysis of limb muscles. but no wrist-drop. She cleaned her paws, purred when spoken to or stroked. Lapped water and milk for the first time since occlusion. Had been fed previously by passing water or milk into her mouth through a tube.

7th day. Walked in a circle, bumping against bars of cage. When placed on floor of room, walked in circle, the size of which was independent of chairs

or tables in the way. Bumped into the objects, probably not because of blindness, but because of lack of control of movements. When put in a cleared space, she walked in a circle as before, going two or three times in the same path, as shown by the marks of her fore-feet, which were dipped in water. Little change in deportment on succeeding days.

12th day. Four kittens were born in the morning. One dead when first seen. Others apparently as vigorous as kittens of a normal mother. Old cat paid no attention to them; apparently totally ignorant of their presence if a few inches away, though they mewed loudly. If a kitten came near enough to touch her nose, she licked it with her tongue, fondled it with her paws when nursing, very much as a normal cat would. If a kitten wandered away, she seemed totally unconscious of its existence, and made no effort to bring it back. The kittens died during the next day.

14th day. Light reflex present in some degree. Cat appeared blind, not closing her eyelids until the eyeball was touched. Refused to walk when placed on the floor. Mewed loudly much of the time, and nothing seemed to pacify her. Appeared deaf to ordinary sounds, but total deafness was doubtful.

15th day. Death by inhalation of chloroform because of puerperal infection. Brain and cord showed no gross lesions on post-mortem examination. A considerable quantity of fat present in omentum and mesentery. Stomach contained undigested meat.

Three stages could be distinguished: (1) a period in which all reflexes, with the possible exception of the light reflex, were present; (2) a prolonged period of convulsions, and (3) a period marked by the loss of intelligence, in which the life of reflexes only was present.

Partial recovery, with paralysis of one fore-leg, and death from accidental strangulation on the ninth day, resulted from an occlusion of eight minutes. There were occasional convulsions following unusually violent attempts to move about in the later period, but there was no loss of intelligence. Another occlusion of twenty-two minutes was followed by paralysis of one fore-leg, but not by any loss of intelligence. Death from pneumonia on the seventh day.

A noticeable feature in all these cases of complete or partial recovery was the prominence of the left nictitating membrane. Frequently also the left pupil was more dilated than the right. We have found that the traction on the ligature under the left subclavian artery usually pulls on the ansa subclavia and the left cervical sympathetic. This injury to the left sympathetic nerve trunk probably explains the inequalities in the eyes.

(*c*) *Death.* After long occlusion, death generally follows in thirty hours or less. No post-convulsive period exists in such cases. One peculiar case comes in this group.

Experiment 83. Occlusion 20 minutes. Symptoms of dementia appeared the third day. Pupils wide and staring; choreic movements of the head. Strong tonic or clonic convulsions followed any attempt at quick or unusual movement. Cat mewed loudly when any one came in sight. Would not eat voluntarily; scratched when milk was passed into her mouth through a tube, but swallowed some of it. Sniffed at a piece of meat held in front of her, but ate none of it. The dementia passed away. On fifth day, cat ate normally, responded to a call, and held her head out to be stroked. Convulsions still followed any complicated or rapid movement. Slow movements were fairly well executed. Kept under observation 9 days, but lost sight of during vacation. Future deportment and circumstances of death, which occurred about 10 days later, unknown.

SUMMARY AND CONCLUSION.

The cerebral circulation was interrupted for periods of three to eighty-one minutes by ligation of the innominate and left subclavian arteries proximal to the origin of the vertebral, in ninety-three cats. Eleven dogs were used in the earlier experiments.

The eye reflexes disappear very quickly and a period of high blood pressure follows the occlusion immediately; vagus inhibition causes cardiac slowing and a fall in blood pressure, followed by a second rise after the vagus center succumbs to anæmia. Respiration stops temporarily (twenty to sixty seconds) after the beginning of occlusion, and then follows a series of strong gasps of the Cheyne-Stokes type, after which it stops until some time after the restoration of the cerebral circulation. The respiratory and vagus centers lose their power of functioning at approximately the same time. Asphyxial slowing of the heart may occur without the agency of the vagus center. The blood pressure slowly falls to a level which is maintained throughout the remainder of the period of occlusion.

The anterior part of the cord and the encephalon lose all function; no reflexes are obtainable. The reflexes of the posterior part of the cord persist; the intravenous injection of strychnine does not affect the anterior part of the cord during the period of occlusion; but does affect the posterior portion of the

cord. There is no secretion of tears or saliva, and the intra-ocular pressure is reduced.

The blood pressure falls still more after release of the cerebra arteries, but soon begins to rise. The respiration returns suddenly, two to sixty minutes after restoration of the cerebral circulation, the first gasp being a strong one. The rate gradually increases until rapid enough for natural respiration. The eye reflexes and intra-ocular tension return more gradually, ten minutes to three hours after restoration of the cerebral circulation. The anterior part of the cord recovers its functions gradually. The first reflexes occur only on the same side as the stimulus, crossing of reflexes, to involve the other side, not occurring till later. As a rule, all reflexes return, and a short period of quiet follows. The anterior part of the cord again becomes irritable to strychnine, but succumbs to its action before the normal part. Spasms, of tonic, clonic, or mixed type, then appear, terminating in (a) death, (b) partial or (c) complete recovery. In partial recovery, disturbances of locomotion, such as walking in a circle, paralysis, dementia, loss of sight, hearing, and general intelligence, characterize the post-convulsive period. After complete recovery, there is a return to normal deportment. No gross lesions of the nervous system, other than a congested appearance of the previously anæmic area, were observed.

Transection of the spinal cord stops the spasms below the level of section. Hemisection of the cord stops the spasms on the same side, below the level of section.

Death, without any return of the reflexes after release of the cerebral arteries, has followed an occlusion of seven and one-half minutes. Respiration has returned after an occlusion of one hour. Five animals have recovered completely after an occlusion of seven minutes or more. Only one animal has recovered completely after an occlusion of fifteen minutes. No animal has recovered completely after an occlusion of twenty minutes.

In Herzen's[26] resuscitation of an animal after several hours of cerebral anæmia, there must have been some anastomotic

[26] Herzen, *Revue médicale de la Suisse romande, 1885*, v, 467.

channels to the brain. Mayer's [27] limit of ten to fifteen minutes of cerebral anæmia, beyond which resuscitation is not practicable, is close to the correct one. It appears to us that, in cases of resuscitation two hours after cessation of the heart-beat, (Prus., loc.cit.) the auricles must have kept up a slow but, in some degree, an efficient movement of the blood through the brain. The truth of this suggestion might be tested by introducing some easily recognized, non-diffusible substance into a vein after the heart-beat ceases to affect a manometer, and later searching for it in the brain and other parts of the body. But, whatever the reason, cerebral anæmia in these cases must have been less complete than in our experiments.

The histological alterations of the cord and brain are now being studied. The results will be published later.

Our thanks are due to Dr. A. Carrel for calling our attention to some of the papers in French.

27. Mayer, *Medicinisches Centralblatt*, 1878, xvi, 579.

TABLE II.

SHOWING LENGTH OF PERIOD OF OCCLUSION, THE TIME OF DISAPPEARANCE, AND THE TIME OF RETURN OF THE REFLEXES AFTER OCCLUSION AND AFTER RESTORATION OF THE CEREBRAL CIRCULATION.

Number of experiment	Period of occlusion in minutes	Time, in seconds after occlusion of disappearance of reflexes				Respiration first ceases (seconds after occlusion)	Last gasp (minutes after occlusion)	Time, in minutes after restoration of cerebral circulation, of return of reflexes				Minutes after restoration when pupil begins to contract
		Corneal	Lid	Light	Dilation of pupil			Corneal	Lid	Light	Respiration	
1	5½	45	—	—	45	—	—	—	—	—	—	—
2	3½	30	—	—	210	—	—	4	—	—	4	1½
3	4⅔	35	—	—	35 200	70	—	15½ 18½	—	—	4¾	2¾ 3½
4	7	20	45	—	180	165	—	—	—	—	‖	—
5	6	80	—	44	165	120	—	18	7¾	11	2⅔	7
6	10	15	—	20	—	—	1¾	—	—	—	81	3½
7	5	20	—	20	—	20	2⅔	23	—	23	9½	9
8	5½	45	—	45	75	*	—	—	—	—	—	—
9	5½	—	—	—	—	27	—	27⅔	—	22⅔	16⅔	21
10	12¾ †	15	15	15	—	20	—	?	—	—	—	—
11	25	—	—	—	—	—	—	52	55	52½	38	20
12	22	—	—	—	—	10	7½	48	48	48	30½	—
13	6¼	10	—	—	165	10	5½	—	—	—	31½	7¼
14	9⅚	27	27	27	240	*	—	11⅛	11⅛	130	—	—
15	5	20	20	20	100	24	2⅚	12½	12½	22¼	½	2⅛
16	14	75	75	75	120	45	5¾	37	32	27	20	11
17	10	25	—	25	—	—	1⅝	25½	25½	32½	5⅛	10½
18	20	75	—	—	75	—	—	39	—	44	5	12
19	25½	40	—	—	120	40	⅓	—	—	48½	27½	7½
20	50	25	25	25	85	25	3⅛	—	—	—	67½	5½
21	81	35	35	35	35	35	11½	**	—	—	—	—
22	16	15	—	—	15	15	2½	44½	—	—	30	5
23	15	—	—	—	110	18	—	—	—	—	8	3
24	30	15	—	—	565	10	8	37¼	36¼	25¼	14¼	10½
25	60 16″	—	—	—	14	14	3⅛	**	**	44½	33⅓	25½
26	81	—	—	—	30	30	3¾	**	**	**	**	**
27	60	—	—	—	120	15	8	**	**	**	**	28
28	51	—	—	—	140	30	10	**	**	**	31	41
29	45	—	—	—	16	44	5½	**	**	**	**	**
30	45	—	—	—	90	65	9	**	**	**	40½	7½
31	41	25	—	—	150	40	7⅓	—	—	—	—	21
32	40	25	—	25	—	15	5	—	—	—	—	5½
33	31	30	—	—	270	35	3	—	32	18	9	3½
34	31	—	—	—	14	35	2	39	—	20	18	23 right eye 86 left eye
35	10	—	—	—	180	120	5	24½	—	—	7	13
36	15	15	—	—	15	50	3½	—	—	—	—	30
37	25¾	—	—	—	—	—	—	**	**	**	**	**

* Persisted throughout occlusion. † Incomplete occlusion.
? Reflexes returned before end of occlusion. ** No return of reflexes

TABLE II—*Concluded.*

Number of experiment	Period of occlusion in minutes	Time, in seconds after occlusion of disappearance of reflexes: Corneal	Lid	Light	Dilation of pupil	Respiration first ceases (seconds after occlusion)	Last gasp (minutes after occlusion)	Time, in minutes after restoration of cerebral circulation, of return of reflexes: Corneal	Lid	Light	Respiration	Minutes after restoration when pupil begins to contract
38	61	—	—	—	45	—	—	**	**	**	**	**
39	8	—	—	—	—	*	—	20	—	—	23	20
40	10′ 7″	—	—	—	15	30	—	89	—	48	11′38″	24
41	8	—	—	—	20	—	—	—	—	—	$7\frac{2}{3}$	$13\frac{2}{3}$
42	8	—	—	—	{ 35 left 90 right	50	2′35″	—	—	—	$8\frac{1}{3}$	{ 8 left 12 right
43	16′35″	—	—	—	25	50	3	—	—	72hrs.	$6\frac{5}{12}$	10
44	$22\frac{1}{3}$	—	—	—	90	—	5	**	**	**	$12\frac{1}{2}$	42
45	$22\frac{1}{4}$	—	—	—	240	—	5	—	—	—	10	28
46	11	—	—	—	180	65	$5\frac{1}{2}$	33	—	—	$22\frac{1}{2}$	33
47	$12\frac{1}{2}$†	—	—	—	—	*	—	—	—	—	—	3
48	$11\frac{3}{5}$	—	—	—	—	—	$7\frac{7}{12}$	—	—	—	4	4
49	$12\frac{1}{4}$	—	—	—	—	—	—	—	—	—	$18\frac{7}{12}$	$16\frac{3}{4}$
50	$14\frac{1}{3}$††	—	—	—	—	—	$3\frac{5}{12}$	**	**	**	**	**
51	8††	—	—	—	—	—	2	**	**	**	**	**
52	$6\frac{1}{12}$	—	—	—	—	—	1	$15\frac{1}{4}$	—	—	$15\frac{1}{4}$	—
53	$6\frac{5}{12}$	—	—	—	—	—	2	**	**	**	**	**
54	$6\frac{5}{8}$	—	—	—	20	—	5	19	—	—	$9\frac{1}{2}$	$9\frac{1}{2}$
55	7	—	—	—	—	—	4	—	—	—	2	$2\frac{1}{6}$
56	8	—	—	—	—	15	⁂	—	—	—	9	—
57	$10\frac{1}{4}$	—	—	—	55	—	1	$26\frac{1}{3}$	—	—	$3\frac{1}{3}$	20
58	$7\frac{1}{2}$	—	—	—	80	—	2	—	—	—	4	16
59	$31\frac{1}{12}$	—	—	—	—	—	2	$47\frac{5}{12}$	—	—	$15\frac{5}{12}$	$23\frac{5}{12}$
60	$14\frac{5}{6}$	—	—	—	—	—	2	—	—	—	26	11
61	$28\frac{1}{2}$	—	—	—	70	—	—	—	—	—	39	$29\frac{1}{2}$

The number of the experiment in these tables does not, in general, correspond to the number of the experiment in the series performed.

* Persisted throughout occlusion.

** No return of reflexes.

⁂ Secondary series of gasps did not occur.

† Incomplete occlusion.

†† Strychnine injected during occlusion

TABLE III.

SHOWING PULSE RATE AT INTERVALS DURING OCCLUSION AND AFTER RESTORATION OF THE CEREBRAL CIRCULATION.

Number of experiment	Minutes from beginning of occlusion	Pulse rate	Minutes after restoration of cerebral circulation	Pulse rate
1	16	171	49	150
	24	180	99	184
	36	171	126	184
	50	138	148	162
	—	—	172	204
	—	—	209	180
	—	—	222	160
	—	—	265	171
	—	—	270	168
	—	—	277	167
2	10	183	31	125
	16	184	50	120
	27	163	104	120
	40	167	122	122
	—	—	162	128
	—	—	186	150
	—	—	202	167
	—	—	246	180
	—	—	271	171
3	3	108	13½	140
	7*	78	25½	150
	9†	168	53½	155
	18⅔	214	67½	156
	26⅔	200	75½	144
	39½	170	109½	138
	—	—	112½	128
	—	—	275½	150
	—	—	313½	184
	—	—	350½	184
	—	—	409½	166
	—	—	485½	192
	—	—	515½	186
	—	—	531½	200
	—	—	582½	158
	—	—	626½	210
	—	—	633½	209
	—	—	645½	209
	—	—	683½	187
4	††	155	27	207
	25	166	393	168
	—	—	408	172
	—	—	448	170
5	3⅚	216	16	166
	13	203	21	150
	20	180	25	162
	25	170	—	—
	30	180	—	—
	35	163	—	—
	40	171	—	—
6	††	157	4	120
	6⅔	180	7	140
	8	180	12	150
	18	165	13½	160
	25	96	37	160
	—	—	276	156
	—	—	590	120
7	—	—	⅛	112
	—	—	6	115
8	††	106	1	112
	1½	120	—	—
9	††	130	—	—
	1½	100	—	—
	2⅛	120	—	—
	4⅝	144	—	—
	10⅝	150	—	—
10	††	156	¾	156
	1½	120	2½	150
	3	156	4	126
	5†	126	6	114
	6½	132	7⅔	96
	8½	180	8½	114
	9	162	14	156
	—	—	17	180
	—	—	18	162
	—	—	20	150
	—	—	52	180
	—	—	109	180
11	††	156	6½	150
	3⅝	132	12½	162
	6½	126	35½	96
	8½	120	37½	108
	—	—	39	72
12	¼	180	2½	138
	½	168	—	—

* Still gasping.
† Last gasp.

†† Immediately before occlusion.
§ First gasp after restoration of cerebral circulation.

TABLE III—*Concluded.*

Number of experiment	Minutes from beginning of occlusion	Pulse rate	Minutes after restoration of cerebral circulation	Pulse rate	Number of experiment	Minutes from beginning of occlusion	Pulse rate	Minutes after restoration of cerebral circulation	Pulse rate
13	††	150	¶	—	17	††	162	1 1/30	156
	3/4	156	26	102		¶	186	10 3/30	72
	4 1/4	156	—	—		3	186	24	144
						5	168	—	—
14	††	150	—	—	18	††	204	—	—
	¶	150	—	—		¶	180	—	—
	1/10	174	—	—		3	192	—	—
	1/4	162	—	—	19	††	210	25	156
	2 1/2	156	—	—		—	—	90	168
	4	162	—	—		—	—	45 hours	204
15	††	168	20 hours	138		—	—	7 days	168
	—	—	6 days	162					
16	††	100	—	—					
	5	180	—	—					
	9	112	—	—					

The number of the experiment in the tables does not, in general, correspond to the actual number of the experiment in the series performed.

†† Immediately before occlusion. ¶ Vagi divided.

STUDIES UPON CALCAREOUS DEGENERATION.

II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES BY FISCHLER'S METHOD, AND A MODIFICATION OF THE SAME.

III. CALCIFICATIONS OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

IV. CALCIFICATIONS OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

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II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES.

Quite recently Fischer[2] has given us a method, a modification of Benda's copper acetate process, of demonstrating the presence of fatty acids in the tissues, either in the free state or in combination with bases in the form of soaps. By this method the fatty acid compounds are converted into the insoluble copper soaps, which in the presence of hæmatoxylin form a varnish-like substance insoluble in weak acids. The chemistry of the combinations is not as yet fully determined, particularly in regard to the reactions with oleic acid. The process, however, is a very precise one, if the tissue be placed in the copper acetate solution immediately after it is removed from the body, when it is found that the soluble and insoluble soaps, along with the fatty acid in the free state, are picked out very clearly. If, however, as Fischer points out, the tissue be placed in water or an aqueous fixing fluid for some time preceding the treatment with copper acetate, a loss (more or less) of the soluble soaps results.

[1]This study was aided by a grant from the Rockefeller Institute for Medical Research.

[2]Fischer, *Centralbl., f. Allgem. Pathol.*, 1904, xv, 913.

I say more or less, for, as I have previously pointed out,[3] what constitute soluble soaps in the test tube are not necessarily such in the body.

The method is determinative only for the fatty acid radical, so that the relative quantities of soaps and the free fatty acids cannot be determined. It is true that Fischer advises fixing one portion of the tissue under examination in a formalin solution, while another portion is fixed in a saturated solution of calcium salicylate in 10 per cent. formalin. Both bits of tissue are then treated with the copper acetate solution and the other two fluids in the usual manner. The tissue fixed in the latter solution will demonstrate the combined presence of the free fatty acids and the soaps, while in the former solution some of the soaps have been dissolved out in the fixing fluid, and it was thought that only the free fatty acid radical would remain. However, the calcium soaps, as Langerhans demonstrated them in fat necrosis, and further as have been shown to be present in all pathological processes of calcification, are also almost insoluble formalin in solution, as are also the albuminous compounds of soaps, so that in the formalin-hardened specimen I demonstrated more than the free fatty acid.

The method, however, is very useful in determining the fatty acid radical, free or combined, in microscopic sections, particularly where calcification is progressing in the tissue. The process further has supported my views regarding the process of pathological calcification, that this constantly occurs through the intervention of fatty acids. It is to be noted, as I have pointed out, that the advanced portions of calcified areas no longer contain fatty acids, for the calcium has in these areas formed a more stable compound with carbonic and phosphoric acids.

A modification of Fischer's method of staining the fatty acids brings this still more clearly to light in such sections. If instead of his second solution, which is a saturated solution of hæmatoxylin in absolute alcohol, I substitute a 60 per cent. saturated alcoholic solution of hæmatoxylin, I avoid disturbing the neutral fats present in the degenerated tissues about

[3] Klotz, *American Journal of Physiology*, 1905, xiii, Supp. No. 1, 21.

calcified areas, and thus allow the use of Sudan III or Scharlach R. to stain these.

The process then becomes in short:

1. Fix the tissue and precipitate the fatty acid radical by treating the sections for 1 to 24 hours in the following solution:

Chromalum, 2.5 grams
Formalin, 4%, 100 c.c.
Dissolve by boiling.
While cooling add
Acetic acid, 5 c.c.
and then
Neutral copper acetate (powdered), 5 grams.

2. Thoroughly wash in water.

3. Cut on the freezing microtome.

4. Stain sections in a saturated solution of hæmatoxylin in 60% alcohol for six hours.

5. Wash sections in water and then treat with the following fluid (Weigert's decolorizing fluid) until the tissue becomes a light brown, while the sites of the fatty acid radical remain black.

Potassium ferricyanide,	2.5	grams
Borax,	2.0	"
Water, distilled,	100.0	c.c.

The sections can then be mounted in Canada balsam.

Blue-black deposits are to be noted in areas where larger quantities of calcium soaps are found.

It is to be remembered that in this method only the copper which is deposited along with the fatty acid stains, and that the structure of the fatty acid compounds as they exist in the tissues is lost. However, this is a minor point, and I am able to determine the smallest particles of the free or combined fatty acid moiety. Now if I wish further to obtain the relation of the fatty acids to the remaining fatty degeneration in the tissue I can stain the sections with Sudan III or Scharlach R.

This combined method of demonstrating the fatty acids and their salts along with the neutral fats is of the greatest service in obtaining a conception of the process of calcification. Thus in the centre of a calcified area the blue-black crystals of the inorganic calcium salts are noted; these are then bordered by an intense black area having outrunners into the surrounding tissue, representing the free fatty acids, the calcium and other soaps; and beyond this area again are to be seen the Sudan stained neu-

tral fats in the degenerating tissues. The tissues which contain fatty acids or their calcium salts are necrotic, and contain no living cells.

This combination of Fischer's fatty acid stain with Sudan III I have used in demonstrating the relation of the fatty acids and their salts to the fatty degeneration in calcified tumors, calcification of the arteries, and phleboliths, and have found all to be of the same character. If in other sections the neutral fats are stained with Sudan III while the calcium salts are blackened with silver nitrate (v. Kossa's method) I can compare the quantity of calcium salts to the fatty acid moiety and roughly determine the amount of calcium soaps present in the tissue. As the process of staining the fatty acids and that of demonstrating the calcium salts both yield black precipitates, I cannot utilize the combined method in the same section. However, as the three staining processes mentioned demonstrate in frozen sections the various stages which calcareous degeneration undergoes, I can follow step by step the stage of fatty degeneration, the production of fatty acids, and the calcium soap formation in all tissues in which pathological calcification is progressing. I have found it advisable to decalcify the tissues in which the fatty acid radical is to be demonstrated, to avoid the confusion with the darkened calcium granules in the finished sections. This is done after applying the copper acetate solution.

CALCIFICATION OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

In a recent number of *Virchow's Archiv*, Scheidemandel[4] discusses the changes occurring in the vessels in rabbits, after the inoculation of adrenalin. Without entering into my own experiments with adrenalin, or touching upon the similarities which these vascular changes bear to arteriosclerosis, it is proper to criticise some of his conclusions which do not conform with the results given by the methods described by me in previous communications.

In his sections of the aorta he finds, when stained with hæma-

[4] *Virchow's Archiv*, 1905, clxxxi, 363.

toxylin and eosin, that between the strands of elastic fibres blue staining granules are deposited in the muscle cells in which the nuclei are still present. In the heavier deposits the whole cells are obliterated and the calcium salts form dark staining bands. The elastic fibres in the areas of calcification, he points out, show a narrowing of their lamellæ, and their staining characters are changed so that they appear granular. In some places he noted tears of the elastic elements. He did not observe any fatty degeneration, nor was he able to determine a calcification of the elastic fibres. He further claims that the elastic fibres are the first to show the degenerative changes in the vessel wall.

In my experience, the deposit of calcium salts within living cells, to the extent that they are stainable with hæmatoxylin, is a very questionable occurrence. The process of calcification is one which does not permit of this, but what I do find, is that in degenerated areas there still exist cells which have not undergone the general necrosis, and in these the nuclei are yet to be seen while the broken down cells about them become calcified. Where living cells exist in calcified areas they do not contain calcium salts to the extent that they will stain with hæmatoxylin. For the detection of finer quantities of calcium, hæmatoxylin stain gives an inadequate reaction, silver nitrate being a more sensitive indicator.

As I have previously pointed out, the process of calcification is preceded by a fatty change, which is followed by a conversion of these neutral fats in the necrotic areas into the fatty acids, and with them the calcium from the blood and lymph forms insoluble soaps.

I have in my possession calcified aortas from rabbits, produced by the inoculation of adrenalin, which present the various stages of the process. The early changes, as has been described by Fischer, are degeneration of muscle fibres. These are shown in my specimens, when stained with Sudan III and hæmatoxlyn, to be fatty degenerations, the fine fat granules lying about the nuclei of the muscle cells. In the more advanced stages the fat granules occupy the greater part of the cells, and the

nuclei show degenerative changes. In this condition of the muscle cells the elastic fibres are packed more closely together (Fischer), which leads to a thinning of the vessel wall. If the calcified vessels be stained with Sudan III, I find that the area of calcification is usually the site of a fat-staining deposit—the calcium soap. I say usually, for in the advanced conditions the calcium soaps have been converted into the calcium carbonate and phosphate. However, the simple application of Sudan III to a calcified vessel may show little or no fatty staining material, although such may be present. If in this case I decalcify the vessel before applying the stain I can then demonstrate the fatty substance. I take it that in such cases the calcium forms a combination with both the fatty acids and the carbonate group, thus, $Ca_n \begin{matrix} \text{Fatty acid} \\ Co^3 \end{matrix}$, and that the combination does not stain with Sudan III until I free the fatty acid from the carbonate. When, however, the calcium forms a combination with the fatty acid moiety alone, as I have noted it in several specimens, then I can demonstrate this fatty acid moiety by means of Sudan III. Hence the calcium, deposited in vessels, exists in the combinations, (a) the pure calcium soap, Ca-Fatty acid, (b) the calcium compound with a fatty acid and a carbonate-radical, $Ca_n \begin{matrix} \text{Fatty acid} \\ Co_3 \end{matrix}$, which stains with Sudan III only after decalcifying, and (c) the calcium carbonate, $CaCO_3$, which under no conditions stains with this stain. (Phosphoric acid may replace the carbonate in each of the above compounds.)

That such a fatty acid moiety exists, can also be demonstrated by Fischer's method of staining the fatty acid radical. But similar to the Sudan stain, if the calcium is in combination with both the fatty acid moiety and the carbonate radical, the specimen must primarily be decalcified before applying the reagents. In these specimens the fatty acids and the neutral fats can be demonstrated in the same section by means of the modification of Fischer's stain that I have previously described.

That calcification of the elastic fibres does occur in vessels is not a new subject (Jores, Matusewicz), and I have several

examples of the process in human arteries. In the arteriers of rabbits where the elastic fibres are very slender it is impossible to differentiate by means of the hæmatoxylin stain the calcareous granules occurring in the debris of the cells from those which occur in the elastic fibres. It is true in the greater number of aortas showing calcification the elastic fibres are the last to be affected, and they can be distinguished as the wavy refractile lines passing through the dark blue mass. However, using silver nitrate in a weak acetic acid solution, to detect calcium deposits, I find, occasionally, the primary fatty degeneration of the elastic fibres, followed by the deposit of calcium salts. By this method the calcium deposit forms a network of black granules about the muscle bundles. It is the exception to find primary fatty or calcareous degeneration in the elastic fibres, and I must consider the degeneration of the muscle bundles as the rule.

What I would particularly emphasize is that the primary degeneration leading to the subsequent calcification in the aortas of experimental animals, is found in the muscle tissue, and that it is only later that the elastic fibres are changed. A fatty degeneration is to be noted in the muscle fibres long before any macroscopic change is seen in the vessel. The sections of such vessels when stained with Fischer's *fuchselin-scharlach* show that the fatty droplets all lie between the strands of elastic fibres.

Scheidemandel points out that in the calcified aortas of rabbits the elastic lamellæ no longer take the elastic stain characteristically, and that the fibres are granular with light or unstained spaces between the granules. These unstained parts are, as he notes, areas of degeneration, and I would further point out that with the use of *fuchselin-scharlach* these spaces are seen to be the sites of fatty degeneration of the elastic fibres, that is, the degenerations occurring in the elastic fibres are also of a fatty nature, and the changes being similar to those found in the muscle cells these fibres may become the site of calcium deposits.

Of rupture of the elastic fibres, in the vessel wall, I have never been able to convince myself, and must say that those that I have seen, outside of complete tears of one or more tunics in

dissecting aneurysms, were artefacts. It is quite evident that, with a degeneration of the muscle fibres, if a rupture of the elastic elements should occur, the intervening space must be filled with blood or coagulated lymph. This is never seen in these specimens. What does happen is that more or less extensive degeneration of the muscle and elastic fibres is found in which these elements no longer take their characteristic stain. It is true that spaces with the ends of the elastic fibres jutting into them from the side are to be seen and that these appear like ruptures of the tissue, but it can be shown with Sudan III and often with the elastic stain that the wavy outlines of the former elastic fibres still stretch through the degenerated area. In human vessels, where degenerations are rarely produced as rapidly as in experimental animals, the new-formed connective tissue fills in the necrotic areas almost as fast as they develop. As Scheidemandel points out, the calcified vessels in rabbits are converted into rigid tubes and no doubt it is in the handling of these fragile sections that the apparent *intra vitam* ruptures in the inner part of the vessel wall result.

In short, the degenerations occurring in the aortas of rabbits inoculated with adrenalin are found chiefly in the muscle cells of the media, and are primarily of a fatty nature, and the inequalities in the staining reaction in the elastic fibres are due also to fatty degeneration of those elements. The calcareous deposits are consequent to the fatty degeneration in that the neutral fats in the degenerated area are converted into fatty acids, which then combine with calcium. The elastic fibres are rarely primarily affected in the degenerative changes, but when they are they may become the primary site of the deposit of calcium. The apparent ruptures of the elastic fibres are either areas of degeneration through which the degenerated fibres still pass, or are artefacts (excluding dissecting aneurysms).

I have to thank Dr. B. Fischer for some of the calcified aortas of rabbits, on which the studies were carried out. A more complete paper dealing with the degenerative processes found in human arteries is to follow shortly.

CALCIFICATION OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

Calcification occurring in the walls of arteries, as one of the degenerative changes in arteriosclerosis, has been extensively described. Particularly well known are the forms of calcification that are found in the intima alone, and which are usually secondary to atheromatous change. Such calcareous deposits have been the subject of discussion ever since arterial disease has been recognized, and later it was shown that these processes remained not alone in the deeper layers of the intima, but advanced into the media.

Jores [5] and Matusewicz [6] have described another form of calcification in arteries, in which isolated patches of the intimal elastic lamina become the site of the deposit. I have met with similar examples, occurring most frequently in the iliac arteries.

Of late Moenkeberg [7] has described a form of calcification that he found in the media of arteries of the muscular type, and noted chiefly in the vessels of the extremities in old people on which clinicians base the diagnosis of arteriosclerosis. His findings are interesting, in that the arterial changes are often confined to these vessels alone, the aorta and its main branches being unaffected. He has found, however, that such degenerative changes do take place in the vessels of the extremities, when more or less arteriosclerosis (intimal disease) is present elsewhere in the body. His statistics show that the femoral artery is most frequently attacked, and that the process having started in one vessel spreads from the primary focus into the branches of the artery. Such a condition as a primary calcification of the media he did not note in any but the vessels of the extremities.

During the routine examination of vessels for degenerative processes in the arterial wall, with the newer technique now at our disposal, I was struck with the frequency with which microscopic calcium deposits were found when the macroscopic examination for the same was entirely negative. I very soon came

[5] *Ziegler's Beiträge*, 1897, xxi, 211.
[6] *Idem.*, 1902, xxxi, 317.
[7] *Virchow's Archiv*, 1903, clxxi, 141.

to the conclusion that the detection of pathological quantities of calcium by the naked eye was not to be relied upon. V. Kossa's method of staining the finer particles of calcium, particularly calcium phosphate, by means of silver nitrate, frequently gave a microscopic picture of calcium salts quite out of keeping with the opinion one formed from the macroscopic examination of the vessel. I have seen specimens in which the aorta was pliable and elastic, with not a trace of calcium salts to the naked eye, while the microscopic section showed the media to be loaded with calcium salts.

Leaving aside for the present the extent and nature of the calcium deposits in the intima, which secondarily invade the media, I wish to discuss the form of calcification found primarily in the media of the aorta and its larger branches, in other words in the vessels of the elastic tissue as distinguished from the muscular type. This form of calcification I have not met with in any other vessels; it differs thus from Moenkeberg's form, which occurs only in arteries of the muscular type.

The age of the patients in which primary calcification of the media of the aorta was found was usually over 65 years; I have encountered three cases under 45 years (aged 39, 41, and 43 respectively). So frequently does a medial deposit of calcium salts occur in old people that it might be ventured that all people over 65 years have it, and that in fully one third of those over 50 years is it present. Sex seems to make no difference.

In the majority of the cases the aorta is the vessel which is attacked, and from here the degenerative process advances into the carotids, innominate, and subclavian arteries. In the latter vessels the condition is as a rule less marked than in the aorta, where the calcareous salts are found uniformly distributed in the media in the different parts of the vessel. No site of predilection was noted in the aorta, but it was found that when one portion of the vessel was affected, calcium salts were found in the media and in other parts also.

It is the common finding that persons over fifty years of age show more or less arteriosclerosis, and hence it would be difficult to draw inferences as to the association of arteriosclerosis at that

time of life with the condition here described. In cases, however occurring before fifty years of age, there was a marked absence of gross arterial disease, so that this form of calcification of the media in the vessels of the elastic tissue type stands apart from what is ordinarily understood as arteriosclerosis. Nor is arteriosclerosis to be found in other parts of the body, though I have noted in some, not all the cases, the presence of calcified areas in the media of the vessels of the extremities as described by Moenkeberg.

Hence it would seem that the condition is a form of senile degeneration of the arterial system in which the media of the aorta and its larger branches are involved. This is further borne out by the microscopic examinations.

Macroscopically, as was stated, the vessel may show no change whatever, and the autopsy protocol invariably contains negative reports, except where a concurrent arteriosclerosis affects the other coats of the vessel. The aorta is elastic, not thickened, and the intima is smooth and glistening, while its surface shows a fine sprinkling of fatty granulations so common in advanced life. These slight intimal changes may be considered as the rule in old age, and although they do not represent a physiological process, they play no leading part pathologically. The naked eye inspection of the media and adventitia discloses no change in their structure.

The microscopic examination of frozen sections shows, particularly in the media, interesting changes.

The media is of normal diameter, and at first sight, in hæmatoxylin stained preparations, one is led to believe that no changes have taken place. A closer examination shows in the central zone of the media numerous clusters of fine dark-stained granules, which lie between the elastic fibres. Thick sections, at times, give the impression that the elastic fibres also contain these granules, an impression seen to be erroneous when thinner sections are examined: they lie only in the degenerated muscle fibres. It is to be noted, too, that the muscle tissue in the areas of the dark-stained granulation contains no nuclei, a point which is likely to be overlooked in a careless examination, as the granulation takes the place of the nuclei. In other such areas, which may be looked on as an early stage of the degeneration, the muscle cells are seen to have imperfect outlines, while the nuclei show stages of fragmentation. Amongst those degenerated muscle cells are to be seen, here and there, other cells which have retained their vitality, and hence give the impression that the granular deposit exists in them.

This is not so; more careful study shows that in no case are living cells to be found having within them this deposit of calcium salts. Nor have I noted in any of my specimens a calcification of the elastic fibres such as is found in the calcified media in the vessels of the extremities.

In one case I noted the presence of new formed blood-vessels in the media. While I take it as normal to have blood-vessels in the outer third of the media, yet the presence of numerous capillaries in the media, even though not accompanied by inflammatory infiltration, must be looked on as a reaction to some irritant. The presence of the blood-vessels in the media diminishes the quantity of calcium salts laid down in its vicinity, and the tissue lying about the vessels is entirely devoid of calcareous salts. And, further, the muscle cells lying near the new formed blood-vessel are not degenerated as are those in other parts. It would seem that with the presence of sufficient nutrition the deposit of calcium salts does not take place. This is further borne out by the absence of any deposit in the media bordering the adventitia, and that lying next to the intima. Thus the deposit of calcium is limited to the middle zone of the media.

The inner zone of the media, I take it, is nourished in part at least from the lumen of the vessel, through the intima. Koester[8] has shown that lymphatic spaces pass from the adventitia to and into the intima, and that inflammatory conditions advancing into the vessel wall pass from the adventitia towards the lumen. Confirming this view that some nutriment passes from within outwards into the walls of the arteries, I have noted that in thrombosed vessels, after a week, the intima contains no living cells of its former tissue, and that this tissue and the inner layers of the media are in a state of fatty degeneration. That is if the blood supply within the vessel is cut off, and an effective state of the vasa vasorum persists, the inner layers of the vessel, including the intima and inner strata of the media, become degenerated, and are only later replaced by connective tissue coming in with the newly formed blood-vessels from the adventitia.

Believing, then, that the degeneration and the later deposit of calcium salts in the media rest in part on the nutrition of that coat, it is seen that the greatest deposit of calcium takes place in the middle zone of the media. In the outer one third, where the media is well supplied with capillaries, and in the inner portions of the media supplied through the intima there is a marked absence of the degenerative process.

[8] *Berlin klin. Wochensch.*, 1876, p. 454.

Moenkeberg found in his cases of calcification of the arteries of the extremities, that the muscle fibres first showed a fatty degeneration. A similar fatty degeneration is also to be seen in the aortas of the cases under description. The fat granules are first found lying about the nuclei of the muscle fibres, while after the degeneration has advanced, and the cell has become filled with the small fat droplets, the nucleus becomes lost. The cell itself is later broken up, leaving the fat granules lying in the interstices of the remaining muscle cells. Thus it is often seen that the intact muscle fibres are surrounded by aggregations of these fine fat particles, which have been described as arising out of the lymph surrounding the cells. With the loss of more or less of the muscle tissue, by this process of fatty degeneration, I find a definite shrinkage at the site where they previously existed; on account of the blood pressure within the vessel, the laminæ are pressed closer together, and the remains of the fatty degenerated muscle fibres are found as clusters of fat droplets in the clefts between the remaining muscle cells. It is further to be noted that these aggregations of fat granules of former muscle cells are found lying next to the elastic fibres of the media, the elastic fibres themselves being unaffected. In none of the specimens that I have noted, in which a calcification of the media of the vessels of the elastic tissue type alone was present, have I seen fatty degeneration of the elastic fibres in the media. Thus it is seen that the disease affects primarily the muscle fibres.

With the modification of Fischer's method of detecting fatty acids and soaps (page 324) I was able, after decalcification of the specimens, to demonstrate that these same areas in the media are areas of advanced fatty degeneration. They give the reactions both for fatty acids and soaps.

That the deposit which I have demonstrated, by the use of silver nitrate, is calcium, is shown by its staining a dark blue with hæmatoxylin, its solution in hydrochloric acid, and the formation of crystals with sulphuric acid.

The best microscopic specimens demonstrating the calcium granules are obtained by the silver nitrate method, in which

the granules are seen to be arranged in clusters parallel to and often closely bordering upon the elastic fibres. The elastic fibres are always to be traced as uninterrupted strands through these areas of degeneration. That the elastic fibres resist degenerative processes to a greater extent than the muscle fibres, I have noted also in other arterial diseases. Thus, too, in the calcified areas of the arteries of the extremities the muscle fibres are the first to be affected, and in the early stage the calcification of these fibres is similar to the process in the muscle fibres in the aorta. The muscle fibres undergo an early fatty degeneration, which is later followed by a deposit of lime salts. As the calcification of the muscle cells advances the granules of lime salts become confluent and form larger masses. In the calcified arteries of the extremities, described by Moenkeberg, it is seen that the outer border of the calcareous deposit has a similar appearance to the calcification in the arteries of the elastic tissue type, the difference between them being that in the former the process goes on much further, attacking the elastic fibres and later forming macroscopic deposits of calcium. Macroscopic deposits of calcium in the media alone of the aorta I have not so far encountered and am led to doubt if they ever occur.

The process of calcification in itself is similar to that found in other degenerative processes. One has to do essentially with a fatty degeneration of the specific cells, followed by death of the cell and a fatty acid stage, in which calcium soaps are laid down. A later conversion of the calcium soaps into the calcium phosphate and carbonate then results.

The intima, as I have said, showed no macroscopic change except in such cases as exhibited a concurrent intimal arteriosclerosis, and in these latter cases the medial disease was equally pronounced in the portions of the aorta lying beyond the intimal plaques. However, in all cases showing calcareous deposit in the media, the intima was found to be fatty, the degeneration being most marked in the deep, or musculo-elastic layer. In the early cases the changes in the intima are mostly a hypertrophy of the musculo-elastic layer, with more or less of a fatty degeneration attending it. The connective tissue layer of

the intima is not thickened, neither does it show any degenerative changes. Similarly no constant characteristics were noted regarding the elastic fibres in the intima, which at times showed an increase in the lamellæ of the internal elastic lamina, while at other times this lamina showed fatty changes. The changes in the elastic fibres in the intima were related more or less to the character and condition of the elastic fibres in the media, being increased when the latter were in a state of granular degeneration.

In conclusion I would say that the calcification of the media of vessels of the elastic tissue type resembles that found in the arteries of the extremities (the vessels of the muscular type) in that the process of calcification is confined to the media, but differs from it in that the deposit of calcium salts does not go beyond the granular stage, and the muscle fibres alone are involved in the process of degeneration, the elastic fibres remaining unaffected. Macroscopically there is no change in the media, while microscopic sections show the media to be loaded with a granular calcium deposit. This process, which stands in close relation with the nutrition of the vessel wall, is a common one in persons over fifty years of both sexes, and almost constant in the aortas of those over sixty-five years of age.